A SYSTEMATIC REVIEW OF THE SCIENTIFIC AND CLINICAL EVIDENCE OF CD73 PLURIPATHOLOGY FUNCTIONS AND THERAPEUTIC OPPORTUNITIES

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EVOLVING HUMAN BIOMARKERS IN THE MANAGEMENT OF CHRONIC DISEASES: A systematic review of the scientific and clinical evidence of CD73 pluripathology functions and therapeutic opportunities

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INTRODUCTION

The development of small-molecule inhibitors of CD73 is an active area of research [Minor M. et al, 2019]. The primary focus of this thesis is to highlight known and emerging functions of CD73 in the human body system.

Ecto-5'-nucleotidase (ecto-5'-NT), also known as CD73, is a glycosylphosphatidylinositol-anchored membrane protein that converts extracellular 5'-AMP to adenosine by dephosphorylating adenosine monophosphate (AMP). CD73 is essential for many physiological and pathophysiological processes, including inflammation, hepatic fibrosis, renal to stop anti-tumor immune surveillance at the level of T and natural killer (NK) cells, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and tumor associated macrophages (TAMs), CD73 converts extracellular adenosine monophosphate (AMP) into immunosuppressive adenosine [Kobie JJ, 2006].

Adenosine (ADO) is a common nucleoside with pleiotropic properties that can mediate both intracellular and extracellular steps in a variety of biological processes. It is normally present in the extracellular area in low quantities. When tissue is injured by hypoxia, ischemia, inflammation, or malignancy, the normally modest concentration of ADO in the interstitial fluids increases rapidly [Morandi F. et al, 2018]. In some areas of inflammation, levels can significantly rise in response to tissue damage or metabolic stress. Increased extracellular adenosine levels are controlled by the adenosine receptors, which are created in part by extracellular ATP (ARs). In order to better understand how the conversion of ATP to adenosine controls inflammation, it is crucial to study the expression of the ecto-5'-nucleotidase CD73 levels on subsets of Th cells. [M.S. Alam et al., 2009]

Nucleotidases, in general, are crucial for preserving the nucleotide pool's balance, and CD73, the family's lone extracellular member, has grown in popularity as an oncology target due to its high expression on both immune and malignant cells. Adenosine produced by CD73 is thought to be a powerful immune suppressor and suppresses the pro-inflammatory response (Chaloin L. et al., 2018). By producing adenosine, an ecto-5'-nucleotidase (NT5E) called CD73 acts as an immunological checkpoint, causing immune suppression and immune escape. In various different cancer forms, elevated tissue levels of CD73 have been associated with poor patient survival and CD73 blockage [Dominici M. et al, 2021]. Understanding the implications of systemic effects of CD73 inhibition is crucial as CD73-mediated signaling-focused immunotherapy and pharmacotherapy become more and more popular [Joolharzadeh P. et al, 2019]. This ecto-nucleotidase helps to maintain immunological homeostasis by converting extracellular ATP to adenosine [Ghafouri-Fard S. et al., 2019]. Adenosine signaling via CD73 controls the inflammatory response. Since then, it has been demonstrated that CD73 drives T-cell differentiation and is diminished in hereditary and acquired immunodeficiencies. CD73's role in T-cell proliferation and function was originally identified in mice in 1989. Immunosuppressive adenosine is produced by CD73 on regulatory T cells (T-regs). et al. [Joolharzadeh P., 2019]. Within the last ten years, CD73's potential as an immunotherapy target has increased significantly. Combination approaches, including as ICIs, adoptive transfer, chemotherapy, and targeted therapy, are the subject of recent studies. According to preclinical research, CD73 and A2AR inhibition both improve anti-PD-1 and anti-CTLA-4 therapy [Harvey J.B., 2020]. Neoplastic cells are recognized and suppressed in large part by the immune system. A few tumor types have been successfully treated with first-generation immunotherapies for many years. However, the majority of these immunotherapies exhibit a lack of significant efficacy or specificity, leading to unfavorable outcomes and having little clinical application. (L. Antonioli et al., 2016)

In recent years, a deeper comprehension of the intricate interactions between the immune system and malignancies has made it possible to pinpoint the essential molecules (such as CTLA-4, PD-1, and PD-L1) controlling these interactions. According to Antonioli L. et al. (2016), this discovery has rekindled interest in

cancer immunotherapeutics that aim to circumvent the pathways used by tumors to resist immune-mediated death. While CTLA-4, PD-1, and PD-L1 suppression is successfully employed in clinical practice, other checkpoint mechanisms are also being actively researched. Ecto-5'-nucleotidase (CD73), one of these new pathways, has been discovered to have a crucial role in promoting cancer immune evasion, making it a prospective target for the creation of novel anticancer immunotherapies. Ecto-5'-nucleotidase (CD73) is a critical player in this scenario because the breakdown of AMP into adenosine creates an immunosuppressed and pro-angiogenic niche in the tumor microenvironment that aids in the development and spread of cancer. Preclinical models have shown that targeting CD73 has positive antitumor effects, and CD73 blockade in combination with other immune-modulating medications [such as anti-cytotoxic T lymphocyte antigen (CTLA)-4 monoclonal antibodies (mAb) or anti-programmed cell death protein (PD)-1 mAb] is a particularly alluring therapeutic option [Antonioli L. et al, 2016].

Clinical trials with anti-CD73 inhibitory antibodies are currently being conducted. For future targeted therapeutics, however, a variety of protective physiological roles of CD73 must be taken into consideration [Minor M. et al., 2019]. Intriguingly, anti-CD73 monoclonal antibodies are currently being tested in multiple clinical studies for the treatment of solid tumors. This is due to the fact that much of the current research on CD73 is in the disciplines of cancer and inflammation. Many malignancies have an overexpression of CD73. Through the suppression of CD8+ T lymphocytes' ability to fight tumors, its expression promotes tumor progression. Targeted CD73 inhibition with a monoclonal antibody (mAb) has been shown to inhibit tumor growth in mice as proof-of-concept. However, more research is needed to determine whether CD73 is a reliable cancer target [Loi S. et al, 2013]. Multiple types of solid tumors and endothelial cells have been reported to overexpress CD73 in the tumor microenvironment where hypoxia is predominating. The prognosis for patients undergoing anticancer therapy is typically bad for this group of cancers, which includes colorectal, breast, bladder, pancreatic, ovarian, leukemia, and melanoma [Chaloin L. et al., 2018]. It has already been shown to some extent how important this ubiquitous protein is from a clinical standpoint and how it affects the outcome and prognosis of malignancies. It has been suggested that the expression of CD73 is associated with tumor growth, angiogenesis, invasion, metastasis, and other characteristics of cancerous tissues, but the mechanisms involved in such associations have not yet been determined [Ranjbar M-A. et al, 2019]. Recently, some studies have revealed that CD73 is a key regulatory molecule of tumor cells and is upregulated in certain malignancies.

A lot of interest has also been produced by the fact that CD73 has been linked to a number of tissue-protective processes, which has revealed fresh information about how it is regulated and functions [Colgan SP, 2006]. For continued development on the fundamental biology, disease processes, and therapeutic targeting of this significant protein, a thorough understanding of the physiological roles of CD73 is needed [Minor M. et al, 2019].

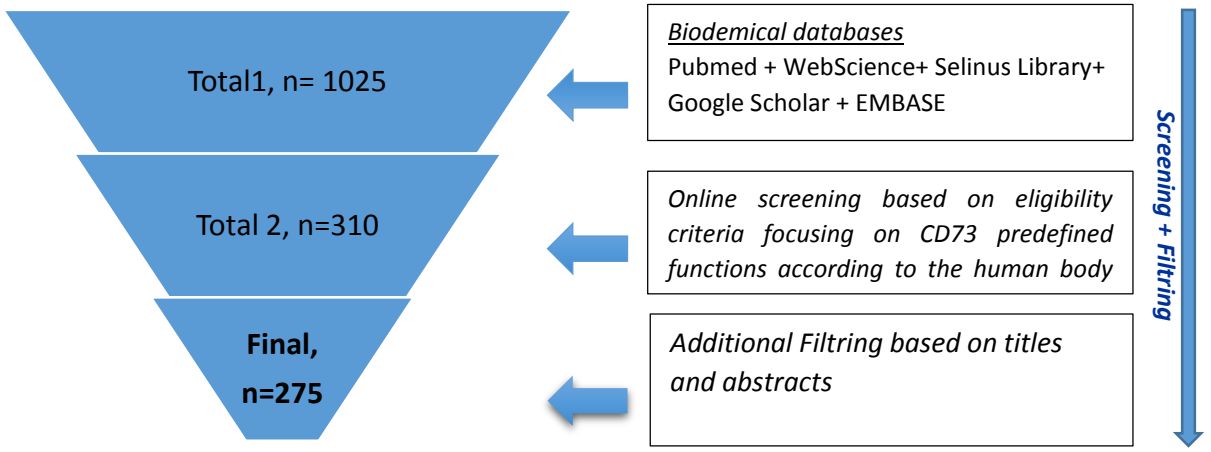
In this review, we aim to investigate the pluridisciplinary role of the ecto-5-prime-nucleotidase, CD73, that applies widely through various organs of the human body system [Joolharzadeh P. et al, 2019]. This membrane-bound extracellular enzyme is indeed found in a variety of tissues, including kidney, liver, colon, brain, lung, and heart; on leukocytes derived from peripheral blood, bone marrow, thymus, lymph nodes, and spleen; as well as on endothelium [Antonioli, L.; et al., 2013]. The hypothesis of this scientific research is therefore to figure out the multifunctional impact of this molecule as expressed in various cells and its potential clinical relevance in practice from diagnosis and prediction through to therapeutic opportunities.

MATERIALS & METHODS

The abundant literature describing Ecto-5'-nucleotidase (ecto-5'-NT), also known as CD73, was te primary source of this systematic review.

Initially, a search of electronic databases produced 1025 possible studies, of which 310 publications matched the inclusion requirements (Figure 1). 35 records were eliminated when those pertinent articles were subjected to screening for eligibility based on relevancy, duplication, and language. Through the use of title and abstract screening, some articles were disqualified. After full-text screening, some further publications were further eliminated. In the end, 275 publications completely satisfied the requirements set forth for this meta-analysis.

Figure 1. summarizes pertinent attributes of the eligible studies.



OUTCOMES & DISCUSSION

The principal objective behind the research presented in this thesis was to determine scientific and clinical evidence of CD73 multifunctional impacts in diseases management from diagnosis, prediction and therapeutic opportunities. This work was performed particularly to address the questions the question whether this membrane-bound extracellular enzyme has limited applications at a preclinical stage or on the contrary its impact as a biomarker is more essential to be widely consider in disease management and therapeutic development.

The rationale behind this question arises from the fact that adenosine is an essential immune system regulator and modulator that can reduce inflammation, which is frequently linked to the onset and spread of cancer [Singh et al., 2019]. Adenosine can help to restore immunological homeostasis by reducing the inflammatory reactions brought on by cell injury (Ohta et al. 2001) and/or cell activation (Huang et al. 1997). Hence our interest in the impact and potential of ecto-5'-nucleotidase (CD73) throughout the human body system.

CHAPTER I: CD73 GENERAL OVERVIEW

1. Biomarkers

Clinical medicine has long made use of biomarkers. Biomarker studies have entered a new era with the introduction of genomics and other developments in molecular biology, and they show promise for the early identification and successful treatment of many diseases. An objectively measured and assessed trait is referred to as a biomarker if it can be used to predict pathogenic processes, normal biological processes, or pharmacologic reactions to a therapeutic intervention. Based on their use in various illness phases, they can be divided into five categories: There are five different types of biomarkers: antecedent biomarkers to determine the likelihood of getting sick, screening biomarkers to detect subclinical disease, diagnostic biomarkers to identify overt disease, staging biomarkers to classify the severity of the disease, and prognostic biomarkers to forecast how the disease will progress in the future, including recurrence, response to treatment, and monitoring treatment effectiveness. The degree or type of exposure to an environmental factor, genetic susceptibility, genetic responses to environmental exposures, markers of subclinical or clinical disease, or indicators of therapeutic response are just a few examples of the characteristics of health or disease that biomarkers can indicate. This chapter will concentrate on the applications of these biomarkers in public health, preventive medicine, diagnostics, treatments, and prognostics, as well as their current state in clinical practice [Xiao-He Chen et al., 2011].

The NT5E gene encodes CD73, a cell surface glycosylphosphatidylinositol (GPI) anchoring protein. Data accumulated indicated that CD73 is a crucial molecule that controls the spread of cancer. The ability of CD73 to produce adenosine from AMP makes it an ectonucleotidase that is associated with the purinergic CD39/CD73/adenosine pathway. Due to its capacity to impede the actions of anti-tumor immune effectors, adenosine is an immunosuppressive chemical involved in tumor immune escape. In addition to its function in allowing tumors to evade the immune system, some reports have provided evidence that CD73 controls tumor cell growth, metastasis, and resistance to treatment by altering signaling pathways like the EGFR/Akt and VEGF/Akt pathways. As a result, CD73 is linked to tumor growth, metastasis, and resistance to therapy. Based on these findings, other research examined the relationship between CD73 expression and survival or disease progression in various solid tumors. Interestingly, CD73 now surfaced as a prospective target to combat the immunosuppressive tumor microenvironment and promote an anti-tumor immune response as well as a potential predictive biomarker [Lafont V. et al, 2018]

2. ECTO-5'-NUCLEOTIDASE (CD73): OVERVIEW, FUNCTION, REGULATION

1. ECTO-5'-NUCLEOTIDASE (CD73): FUNCTION AND REGULATION

1. Cluster of differentiation 73, ecto-5'-nucleotidase (CD73)

Differentiation cluster Ecto-5'-nucleotidase (CD73), a 70kD member of the 5'-nucleotidase family, is anchored to the cell surface by glycosyl-phosphatidylinositol (GPI). It is both a membrane-bound and soluble molecule that hydrolyzes extracellular nucleotides into membrane permeability nucleosides. CD73 is a helpful lymphocyte differentiation marker since it is widely expressed on vascular endothelium and some lymphocyte subpopulations [Airas L. et al, 1997]. The enzymatic dephosphorylation of nucleoside 5'-monophosphates, such as adenosine 5'-monophosphate, is known as 5'-nucleotidase (5'-NT) (AMP). This action can take place inside the cell or in the extracellular environment and is essential for purine salvage and purinergic signaling [Minor M. et al., 2019]. A purine salvage enzyme called ecto-5-sp-nucleotidase (5-sp-NT, CD73) is present on the surface of a subset of human lymphocytes, a few leukemias and lymphomas, as well as numerous other cell types. The production of extracellular adenosine for adenosine receptor engagement, lymphocyte maturation and activation, and providing chemotherapy resistance to some tumor cell lines are additional roles for purine salvage

that have been hypothesized. 1995 [HANSEN K.R.]. The primary enzyme responsible for producing extracellular adenosine from AMP is ecto-5’-nucleotidase, which is represented by the NT5E gene. Following the identification of three distinct antibodies that immunoprecipitated a 69-kDa protein from the human myeloma cell line U266 and bound similarly to human lymphocytes, this enzyme was given the designation cluster of differentiation (CD) 73 in 1989 [Minor M. et al, 2019]. When the ligand is engaged, it conveys signals that activate T cells, just like many other GPI-anchored molecules. Human T-cells utilize CD73 as a co-stimulatory molecule for both proliferation and activation, and it also seems to affect lymphocyte adherence. Additionally, the activity of ecto-5'-nucleotidase itself is a crucial anti-inflammatory mediator since it changes extracellular AMP into adenosine, a powerful anti-inflammatory trigger [Airas L, 1997].

Ecto-5'-nucleotidase, also known as CD73, is a glycosylphosphatidylinositol-anchored glycoprotein that is widely expressed and responsible for converting extracellular adenosine 5'-monophosphate to adenosine [Minor M. et al., 2019]. The rare condition arterial calcifications owing to CD73 deficiency is brought on by missense loss-of-function mutations in the NT5E gene, which codes for CD73. Water transport is an essential part of epithelial cells' physiological processes. This function is carried out by epithelial-lined mucosal tissues like the colon and lung through a planned set of ion transport events [Colgan SP, 2006]. In addition to its direct participation in human disease, CD73 has important roles in tissue homeostasis and pathology in a variety of organ systems, according to cellular and animal model studies. In the setting of the central nervous system, CD73 has antinociceptive properties, guards against inflammatory damage, and also helps to slow the aging process by reducing cortical plasticity [Minor M. et al., 2019].

According to Minor M. et al. (2019), CD73 is a complex molecule that experiences N-linked glycosylation, homodimerization via disulfide bonds, and membrane attachment via a glycosylphosphatidylinositol (GPI) anchor. Ecto-enzymes located on the cell surface quickly digest circulating or locally released nucleotides. Ecto-50-nucleotidase (CD73), a membrane-bound glycoprotein coupled to glycosyl phosphatidylinositol (GPI), hydrolyzes extracellular nucleoside monophosphates to produce bioactive nucleoside intermediates [Colgan SP, 2006].

Numerous studies have identified crucial roles for CD73 that are distinct from its AMPase activity, such as [Minor M. et al., 2019]:

1) by functioning as a costimulatory signaling molecule to activate T cells,

2) promoting integrin clustering by enabling lymphocyte adhesion to the endothelium,

3) making leukemic cells resistant to apoptosis using mechanisms that depend on the GPI-anchor,

4) causing endothelium and lymphocyte protein phosphorylation in response to antibody ligation, and

5) preventing the internalization and aggregation of membranes that lead to the spread of breast cancer cells.

Interferon-alpha is a crucial in vivo modulator of CD73 in the endothelial microenvironment as a method of modulating the inflammatory response, according to other intriguing investigations with CD73 antibodies [Sotnikov I, 2010]. Furthermore, it is known that CD73 regulation and function vary depending on the type of cell in terms of phospholipase sensitivity, membrane shedding, and capacity to activate intracellular signaling in response to antibody stimulation [Minor M. et al., 2019]. Physiology and pathophysiology are largely influenced by nucleotides and nucleosides. A group of ecto-nucleotidases metabolize extracellular nucleotides through controlled phosphohydrolysis. Ecto-50-nucleotidase (CD73), a glycosyl phosphatidylinositol-linked membrane

protein present on the surface of numerous cell types, is principally responsible for converting adenosine 50-monophosphate into extracellular adenosine [Colgan SP, 2006].

The maintenance of tissue barriers, hypoxia, ischemia preconditioning, and inflammation are among the several physiological responses controlled by adenosine receptors that have been shown to be strongly correlated with CD73 activity [Minor M. et al., 2019]. Adenosine 50-monophosphate (AMP), which is converted to adenosine by surface-bound CD73, can be internalized by dipyridamole-sensitive carriers or activated by one of four types of G-protein coupled, seven transmembrane spanning adenosine receptors (AdoR). Numerous cell types have been shown to express more than one isoform of the adenosine receptor, which is one of many cell types that express adenosine receptors. Similarly, it has been demonstrated that surface AdoR activation controls a variety of physiological outcomes. ATP and ADP are phosphohydrolyzed by CD73 to produce AMP [Colgan SP, 2006].

Adenosine monophosphate (AMP) is converted to adenosine by the ectoenzyme CD73 (Ado). A1R, A2AR, A2BR, and A3R are the four types of G protein-coupled adenosine receptors (ARs) that extracellular adenosine can activate. A1R/A3R inhibit and A2AR/A2BR activate adenylate cyclase (AC). Phospholipase C (PLC), phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and ion channels are additional mechanisms by which ARs can signal. Alternately, equilibrative nucleoside transporters can take up adenosine intracellularly (ENT). According to reports, CD73 signaling that is adenosine-independent happens through interactions between proteins, the glycosylphosphatidylinositol anchor, and other processes [Minor M. et al., 2019].

2. CD73 expression

The folling cells are known to be associated with CD73 expression (see Table 1):

- ☐ Effector T Cells
- ☐ T Regulatory Cells
- ☐ Natural Killer Cells
- ☐ Myeloid-Derived Suppressor Cells
- ☐ Tumor-Associated Macrophages
- ☐ Dendritic Cells

Th cells may play a role in local adenosine buildup and the regulation of inflammation, as shown by the expression of CD73 by Th cells and the enrichment of this enzyme in T-reg in humans. Furthermore, altered T-reg function, increased stomach inflammation, and decreased levels of colonization on challenge were linked to decreased adenosine synthesis in CD73 animals. Together, these findings lend credence to the idea that adenosine synthesis and its anti-inflammatory properties may play a role in the duration of Helicobacter infection. Th cells that express CD73 contribute to local adenosine buildup and gastritis attenuation, which may encourage prolonged infection [Alam M.S. et al, 2009].

Numerous researches have used immunohistochemistry staining (IHC) to identify CD73 gene expression, and some of these investigations have used combined gene and protein detection. The median expression of CD73 staining was 50.77 percent among the studies that were designated as CD73 positive, while the range of CD73 expression in solid tumors was 26.4 to 74 percent. For the majority of investigations, tumor cell labeling was utilized to measure CD73 expression [Wang R., 2017]. Ecto-5'-nucleotidase (CD73) is expressed by regulatory T cells (T-reg), and by producing adenosine, they help the enzyme perform its inhibitory role [Alam M.S. et al, 2009]. NT5E/CD73 is widely expressed and controls important processes in a number of organ systems by acting on particular cell types. Inhibiting endothelial permeability, enhancing lymphocyte-endothelium interactions,

inhibiting macrophage- and mesenchymal cell-mediated inflammation, hyperpolarizing and relaxing smooth muscle cells, and anti-nociception via modulation of neuronal activity are some of the major cell type-specific functions of CD73, according to Minor M. et al. (2019). Normal cell maturation results in an increase in CD73 expression and enzymatic activity. Adenosine promotes CD73 expression and enzymatic activity in a paracrine manner, making it one of the recognized activators. Adenosine is the byproduct of CD73's own enzymatic activity. This component does not appear to be used by IFN-, which instead appears to exercise its effects through indirectly stimulating other mediators that then result in the up-regulation of CD73 [Niemelä j. et al., 2004].

It is well known that human regulatory T helper cells express CD73, which inhibits the generation of proinflammatory cytokines and the gastritis that Helicobacter felis causes in mice. It has been proven that CD73 regulates Helicobacter felis-induced gastritis and colonization through being expressed on human T helper (Th) cells. In instance, CD25+Foxp3+ T-reg from peripheral blood or stomach mucosa have been demonstrated to express CD73, according to studies on CD4+ T cells. All Th cells had significantly more CD73 expression after activation. The production of interferon was increased when CD73 was inhibited. While T-reg from CD73 animals did not prevent gastritis, the gastritis in H. felis-infected CD73 mice was noticeably worse than that in wild-type mice and was accompanied by higher levels of proinflammatory cytokines and less bacterial colonization [Alam M.S. et al, 2009].

Salivary gland tumors that metastasize to lymph nodes exhibit higher levels of CD73 expression than those that do not [Ranjbar M-A. et al., 2019].

Table 1. CD73 expression on various cell types and tissues and its prognostic finding *(page 10) – Adjusted from the published source: Alam M.S. et al, 2009. Extracellular adenosine can be eliminated through enzymatic inactivation, cellular absorption, or receptor binding. Adenosine is converted to inosine by the enzyme adenosine deaminase (ADA), which can take place either extracellularly or intracellularly. The biological actions of the adenosine receptors include context-dependent pro- or anti-inflammatory activities that try to maintain or restore tissue homeostasis.*

Table 1. CD73 expression on various cell types and tissues and its prognostic finding

Cell type	Tissue or cell type	Origin	Reported prognostic finding
Monocyte	Peripheral blood post-infarcted myocardium	Human Swine	Mesenchymal Stem Cells Induce Expression of CD73 in Human Monocytes *in vitro* and in a Swine Model of myocardial Infarction *in vivo*. Positive ADO loop leads to attenuation of inflammation and promotes the regeneration of the damaged myocardial tissue
	Monocytes in the inflamed joint	Murine	CD73 expression is associated with the suppression of inflammation in rheumatoid arthritis
Neutrophil	Neutrophils in the inflamed joint	Murine	CD73 expression is associated with the suppression of inflammation in rheumatoid arthritis
Dendritic cell	Skin	Murine	Production of Extracellular Adenosine by $CD73^+$ Dendritic Cells Is Crucial for Induction of T cell Anergy and Tolerance in Contact Hypersensitivity Reactions
MDSC	MDSCs generated from mouse hematopoietic progenitor cells (*in vitro*)	Murine	Generation of ADO by CD73 may promote MDSC expansion and facilitate their immunosuppressive activity
	Peripheral Blood from advanced melanoma patients	Human	High baseline levels of CD73 on MDSCs negatively correlate with Overall Survival and Progression Free Survival
Macrophage	Alveolar macrophages	Murine	CD73 expression in the lung tissue contributes to radiation-induced lung fibrosis
	Peritoneal macrophages	Murine	CD73 regulates anti-inflammatory signaling between apoptotic cells and endotoxin-conditioned tissue macrophages and is required to limit neutrophil influx in a peritonitis model
NK cells	Peripheral blood 2–5% $CD73^+$ NK cells	Human	ADO induces T cell suppression
	Upregulation of CD73 upon exposure to MSC (*in vitro*)	Human	$CD73^+$ NK cells have the potential to regulate NK cell activation in an autocrine or paracrine manner
B cells	Subpopulations of murine memory B cells, germinal center B cells	Murine	ADO signaling is prominent in the mature germinal center and required for establishment of the long-lived plasma cell compartment
	Peripheral blood and tonsil	Human	Dependence of Immunoglobulin Class Switch Recombination in B Cells on Vesicular Release of ATP and CD73 Ectonucleotidase Activity Common variable immunodeficiency (CVID) patients with impaired class-switched antibody responses are selectively deficient in CD73
	Colon B cells	Murine	B cell CD73/CD39/adenosine mediates immunosuppression in DSS-induced colitis
T cells	Th1, Th2, Th17, Treg in normal, and tumor tissues	Murine human	CD73 may favor cell homeostasis, memory survival, and differentiation
		Human	$CD73^+$ T cells infiltrate into breast and ovarian tumor tissue
		Human murine	ADO induces immunosuppression
		Murine	CD73 expression on extracellular vesicles derived from Treg contributes to their regulatory function
		Murine	CD73 expression in the lung tissue contributes to radiation-induced lung fibrosis
Fibroblasts	Cancer-associated fibroblasts in High-grade serous ovarian cancer (HGSC)	Human	High CD73 expression on CAFs is associated with worse prognosis
	Cancer-associated fibroblasts in bladder cancer	Human	High CD73 expression on CAFs is associated with worse prognosis
Epithelial cells	Retinal pigment epithelial cells	Murine	CD73 expression is associated with the suppression of conventional CD4 cell proliferation
	Renal epithelial cells	Murine	CD73 expression on proximal tubular epithelial cells Is critical in renal ischemia-reperfusion injury protection
Endothelial cells	Bladder cancer	Human	High CD73 expression is associated with better survival in non-muscle-invasive BC (NMIBC) and muscle-invasive BC (MIBC) tumors
Mesenchymal stem cells (MSC)	Experimental autoimmune uveitis (EAU)	Murine	Inhibition of T-cell proliferation

i. Effector T Cells and T Regulatory Cells

Ecto-5'-nucleotidase (CD73) is a protein that has been linked to immune suppression, tumor progression, and cancer patients who respond well to anti-PD-1 immunotherapy. Less is known about CD73 expression in other immune cell groups, despite the fact that regulatory T cells can express CD73 and block T cell reactions by generating adenosine. In breast cancer patients, the frequency of these CD73-positive NK cells was connected with bigger tumor size, and tumor-infiltrating NK cells upregulate CD73 expression. Additionally, compared to NK cells that were CD73-negative, LAG-3, VISTA, PD-1, and PD-L1 expression was significantly higher in NK cells that were CD73-positive [Yong Neo S. et al, 2019]. The main component of adaptive immunity, T cells, aid in the defense against infections and malignancies. Through an increase in intracellular cAMP levels, adenosine can affect the function of T lymphocytes. Additionally, clonal growth of immunologically active antitumor T lymphocytes is prevented by adenosine-mediated suppression of IL-2 production in the tumor microenvironment. According to Jin et al., overexpression of CD73 on tumor cells increased T-cell death in vitro and decreased the antitumor effect of T-cells in vivo, although both deficiencies could be corrected by suppression of CD73 expression. Furthermore, Ryzhov et al. discovered that CD73 in tumors was important for myeloid-derived suppressor cells (MDSCs), a population of immature myeloid cells that have the capacity to inhibit T-cell activation [Zhang H-Z. et al., 2014].

On effector T cells, A2AR is increased during inflammation. Its activation prevents the production of cytokines such tumor necrosis factor (TNF), interferon-gamma (IFN), and interleukin-2 (IL-2) as well as effector T cell proliferation, cytotoxic activity, and cytokine. While T regulatory (T-reg) cell activation via the A2AR enhances T-reg cell growth and immunosuppressive action. Mechanistically, a self-reinforcing loop connects these actions. Effector T cell activity is suppressed by CD73 on T-regs because it produces extracellular adenosine and activates A2AR on effector T cells. Additionally, extracellular adenosine activates T-regs' A2AR, boosting their growth and activity. Unlike mouse T-regs, human T-regs hardly ever express cell surface CD73. The extracellular adenosine is thought to be produced by exosomes or adjacent cells that express the CD73 protein. Immune cells, stromal cells, epithelial and endothelial cells, cancer cells, and exosomes all express CD73 [Harvey J.B., 2020]. Less is known about the expression of these ectonucleotidases in conventional T cells and NK cells, despite the fact that regulatory T cells have been found to express the ectonucleotidases CD39 and CD73 and suppress T cell responses by producing adenosine. T cells upregulate CD39 when exposed to mesenchymal stem cells (MSCs), which causes activated T cells to be suppressed by more extracellular adenosine being produced (Yong Neo S. et al., 2019).

T-regs are essential for extending cardiac transplant life. ERCs that express CD73 greatly enhanced the population's fraction of T-regs, according to earlier studies. However, inhibiting CD73 expression on ERCs significantly lowers the T-reg population. The metabolism of ADO may possibly be responsible for the process. According to Schenk et al., Th17 cell polarization was mediated by the ATP receptors P2X7R. It suggests that the elevation of T-regs and the reduced ATP content in the immediate microenvironment are connected. However, after the adoption of the ERCs, the CD73 function known as ADO genesis would increase T-regs by sequentially reducing the concentration of local ATP. Additionally, it has been shown that mouse T-regs can independently lower the amount of ATP in their immediate environment by expressing CD73, which in turn encourages T-reg differentiation [Wang H. et al., 2020].

ii. Natural Killer Cells

Natural killer (NK) cell A2AR activation prevents NK cells from maturing, proliferating, activating, producing cytotoxic cytokines (such IFN- and TNF-), and killing target cells. While NK cell maturation, proliferative capability, and cytotoxic activity are restored by genetic deletion, pharmacological blockage of A2AR, or respiratory

hyperoxia, which enhances tumor growth control, delays tumor starts, and suppresses tumor spread. Strategies to boost antitumor immunity may include CD73 and/or A2AR inhibition, more oxygen, and treatments that encourage NK cell activation. By reversing hypoxia-extracellular adenosine-mediated immunosuppression, whole-body exposure to 60% oxygen slows the growth of tumors [Harvey J.B., 2020]. Ecto-5'-nucleotidase (CD73) is a protein that has been linked to immune suppression, tumor progression, and cancer patients who respond well to anti-PD-1 immunotherapy. Less is known about CD73 expression in other immune cell groups, despite the fact that regulatory T cells can express CD73 and block T cell reactions by generating adenosine. In breast cancer patients, the frequency of these CD73-positive NK cells was connected with bigger tumor size, and tumor-infiltrating NK cells upregulate CD73 expression. Additionally, compared to NK cells that were CD73-negative, LAG-3, VISTA, PD-1, and PD-L1 expressed considerably more frequently in NK cells that were CD73-positive. Upon interaction of 4-1BBL on tumor cells, NK cells transfer CD73 in intracellular vesicles to the cell surface and the extracellular environment via actin polymerization-dependent exocytosis. These CD73-positive NK cells go through transcriptional reprogramming, upregulate the production of IL-10 through STAT3 transcriptional activity, and decrease the proliferation of CD4-positive T cells and the production of IFN-. Together, these findings show that malignancies can use NK cells to evade defense mechanisms and that the tumor microenvironment creates an inducible population of NK cells with immunoregulatory features based on CD73 expression. 2019 [Yong Neo S. et al.]

Extracellular adenosine levels and the expression of the genes for CD73, A2AR, and A2BR are demonstrated to decrease in preclinical trials and improve antitumor immunity. Hypoxia-inducible factors (HIFs) work together to promote extracellular adenosine/adenosine receptor signaling for reducing inflammation. HIFs are highly associated with raising CD73, A2AR, and A2BR gene expression. It's interesting to note that recent research has shown tumor cells can rewire NK cells to become immunosuppressive. Adenosine receptors are not engaged in the effects, indicating that additional mechanisms may be at play that do not necessarily entail the creation of extracellular adenosine [Harvey J.B., 2020]. Upon interaction of 4-1BBL on tumor cells, NK cells transfer CD73 in intracellular vesicles to the cell surface and the extracellular environment via actin polymerization-dependent exocytosis. These CD73-positive NK cells go through transcriptional reprogramming, upregulate the production of IL-10 through STAT3 transcriptional activity, and decrease the proliferation of CD4-positive T cells and the production of IFN-. We conclude that CD73 expression defines an inducible population of NK cells with immunoregulatory features inside the tumor microenvironment and that malignancies can hijack NK cells as a means of evading immunity [Yong Neo S. et al., 2019].

Although it is believed that NK cells help to eradicate tumors, research has shown that in situations of acute infection and inflammation, NK cells can change into regulatory cells that produce adenosine and IL-10. In the setting of cancer more recently, CD56+CD3- cells in patients with ovarian cancer inhibited the proliferation of T cells as seen in an ex vivo expansion of tumor-infiltrating lymphocytes (TILs). It has been shown that NKp46 interaction caused the suppression, however the exact processes by which NK cells suppress are still unknown. Furthermore, it is yet unknown how ordinary NK cells can change their phenotype in order to suppress other TIL populations and aid tumor immune evasion. 2019 [Yong Neo S. et al.]

iii. Myeloid-Derived Suppressor Cells and Tumor-Associated Macrophages

The ectonucleotidase activity of CD73, which is increased on CD11b+ CD33+ peripheral blood and tumor-associated myeloid-derived suppressor cells (MDSCs), suppresses the activity of T cells and NK cells. Patients with colorectal cancer are described as having granulocytic MDSCs with high CD73 expression. It was discovered that these cells had strong immunosuppressive characteristics, such as increased PD-L1 expression and activity that could be reduced by inhibiting CD73. CD11b+ Gr1+ MDSC proliferation and intratumoral accumulation are favorably encouraged by A2BR activation. MDSCs that are CD11b+ Gr1+ express a lot of CD73, which inhibits T

cell proliferation. Activating A2BR on myeloid progenitors by producing extracellular adenosine, CD73 is also thought to aid MDSC proliferation. Therefore, inhibiting A2BR lessens the immunosuppression and tumor-associated increase of CD11b+ Gr1+ MDSCs. Cancer cells produce extracellular adenosine, which can attract tumor-associated macrophages (TAMs), and whose endonuclease activity, in conjunction with CD73 expression on other cells in the tumor microenvironment, further contributes to extracellular adenosine-mediated immunosuppression in tumors, such as inhibiting antitumor CD4+ T cell proliferation. 2020 [Harvey J.B.]

iv. Dendritic Cells

Dendritic cells (DCs) serve as the link between innate immunity and adaptive immunity and are a crucial part of the immune system. High levels of adenosine bind to the A2B receptor on DCs, which skews DC differentiation toward a certain cell population (i.e., adenosine-differentiated DCs). According to Novitskiy et al., adenosine-differentiated DCs produced significant quantities of VEGF, IL-8, IL-10, COX-2, TGF-, and IDO, which are angiogenic, immune suppressor, and tolerogenic factors. Adenosine also significantly reduced the production of IL-12, a crucial anticancer cytokine, via binding to the A2A receptor on DCs [Zhang H-Z. et al., 2014]. To build up antitumor immunity, these cells (DCs) deliver tumor antigens to cytotoxic T lymphocytes. Due to chemokine receptor downregulation brought on by A2BR activation, DCs become less mobile and more tolerant to the tumor microenvironment. Inhibited allostimulatory activity and high amounts of angiogenic, immunosuppressive, and tolerogenic factors, such as vascular endothelial growth factor (VEGF), IL-8, IL-6, IL-10, TGF-, and idoleamine 2,3-dioxygenase, are two effects of A2BR activation on DCs, for instance. These cells are unable to activate T helper type 1 (Th1) and CD8+ T cells. A2BR blockade promotes DC activation with increased CD86 expression on CD11b-DCs, increases CD4+ and CD8+ T cell IFN-production, and increases tumor cell IFN- and CXCL10 expression, which supports the therapeutic potential of A2BR antagonists in enhancing antitumor immunity [Harvey J.B., 2020]. A2BR binding also inhibits monocyte differentiation to DCs.

The limits of employing murine model systems for the investigation of human disease are highlighted by the disparities between the phenotypes of CD73-deficient mice and humans. In order to get valuable insights into vascular calcification, matrix dysregulation, aneurysmal illness, arthritis, and pain modulation, it is imperative that an adequate model be created. As the medial layer is the primary site of ACDC pathology, in vitro studies should focus on these cells. However, given the influence of CD73 and adenosine signaling on modulating extracellular matrix and inflammatory cell differentiation and signaling, it is crucial to create co-culture or three-dimensional organoid models to examine the interactions between various cells and matrix elements [Joolharzadeh P. et al., 2019].

3. Ectonucleotidases and Adenosine

Adenosine (ADO), a purine nucleoside produced by the action of the CD73 enzyme, plays a part in numerous physiological and pathological occurrences. It is connected to the regulation of the host's defense against infection. In part, CD39 (nucleoside triphosphate dephosphorylase) mediates the dephosphorylation of ATP to ADP and then to 5'-AMP, the substrate for CD73 (ecto-5'-nucleotidase), which catalyzes the terminal reaction to convert 5'-AMP to adenosine [Alam M.S. et al, 2014]. This causes adenosine to accumulate in inflamed It attaches to particular cell surface receptors. A1R, A2aR, A2bR, and A3R are the four distinct subtypes of G protein-coupled adenosine receptors that have been cloned to far. Through raising extracellular adenosine levels, ecto-5'-nucleotidase activity is demonstrated to be a key mediator of the anti-inflammatory impact of methotrexate and sulfasalazine in vitro and in vivo in the mouse air pouch inflammation model. By inhibiting neutrophil degranulation, adenosine controls the detrimental effects of inflammation by limiting leukocyte adhesion to endothelium. It also functions as an anti-inflammatory drug by binding to A2 and A3 receptors. Additionally, adenosine reduces eosinophil migration by activating the A3 receptor. By activating the endothelium A2bR,

adenosine, which is created from AMP produced by neutrophils, improves endothelial barrier function. This AMP-induced promotion is CD73-mediated, and cAMP intracellular levels rise as a result. Recent research has demonstrated the importance of the A2a receptor in reducing in vivo systemic and tissue-specific inflammatory responses. By activating particular ADO receptors (ADOR), particularly A1, A2A, A2B, and A3, which differ in function and tissue distribution, ADO functions as a warning signal. ADO may cause a variety of cellular reactions that are intended to reestablish tissue homeostasis when it interacts with various receptors. ADO is one among them and can control immunological and inflammatory responses to prevent tissue damage and advance the healing process. NK cells, dendritic cells, monocytes, and macrophages are only a few of the immune system's cell types and subsets that can have their functions inhibited by the immunosuppressive chemical ADO (Morandi F. et al., 2018). The promotion of immunosuppression is known to be aided by CD73 and Adenosine Receptor Activity [Harvey J.B., 2020]. Adenosinergic ectoenzymes that are expressed on the membrane of many cell types cause the production of ADO. By metabolizing either NAD+ or ATP (canonical route), ADO can be produced (alternative pathway). Ectonucleoside triphosphate diphosphohydrolase (NTPDase) CD39 initiates the canonical route by converting ATP to ADP. The latter molecule can also be converted by CD39 into AMP, which is entirely dephosphorylated to ADO by the 5-nucleotidase (5-NT) CD73. Since ADO produced by these ectonucleotidases interferes with anticancer immune responses, CD39 and CD73 have lately been suggested as new targets for checkpoint inhibitors [Morandi F. et al, 2018]. By reducing inflammation brought on by cardiac injury, acute lung injury, intestinal ischemia-reperfusion injury, and inflammatory bowel disease, extracellular adenosine protects tissues. Extracellular adenosine is used by tumors as a form of protection for cancer cells. Tumors have an accumulation of extracellular adenosine, which inhibits cytotoxic T cells and natural killer cells. Numerous studies using syngeneic and/or spontaneous tumor models demonstrate that genetic deletion of CD73 or A2AR or pharmacological blockade of these receptors significantly reduce tumor growth and metastasis; this effect is primarily brought on by the restoration of antitumor immunity [Harvey J.B., 2020].

According to Morandi F. et al. (2018), both intracellular and extracellular pathways can control ADO levels.

- ☐ Nucleoside transporters that can transport ADO inside of cells, such as equilibrative nucleoside transporters (ENT1, ENT2, ENT3, and ENT4) and concentrative nucleoside transporters (CNT1, CNT2, and CNT3);
- ☐ Adenosine deaminases (ADA1 and ADA2), which can convert ADO into inosine and are expressed by many cell types. However, through interacting with the A2a receptor, inosine can potentially have immunosuppressive effects.

Different populations of regulatory cell surfaces have adenosinergic ectoenzymes. T-regs are CD4+CD25 FOXP3+ cells that exhibit high amounts of CD39 and CD73. After interacting with ADORA2A, the ADO generated is thought to play a crucial role in reversing the effectsor T cell activities. ADO deaminase activity allows effector T cells to offset the inhibitory impact (ADA). The glycoprotein CD26, which is attached to the cell surface, is the host for ADA, which is in charge of adenosine breakdown. Additionally, CD56 CD16 NK cells have a variety of functions in the control of immune response [Morandi F. et al, 2018].

The presence of AMP is a requirement for adenosine production. It can either be irreversibly degraded into adenosine by the enzyme ecto-5'-nucleotidase or regenerated into ATP by ecto-nucleotidase kinase events. AMP is a crucial intermediate metabolite of ecto-enzymatic ATP metabolism. Because CD73 acts as a master switch to control the transition from the ATP-consuming/adenosine-producing pathway to the ATP-generating pathway, the concentration of AMP is not a limiting factor. Instead, anytime more AMP is required for adenosine production, less ATP is produced. Furthermore, pathological circumstances such tissue damage and hypoxia cause large levels of AMP to be released [Niemelä j. et al., 2004]. The other enzyme in the traditional pathway for the formation of ADO, ecto-5'nucleotidase (NT5E; CD73), is actually produced by responder T cells rather than Tr1 cells. B lymphocytes are also accompanied by regulatory cells. ADO is produced by a CD73+ subset of B cells that has regulatory characteristics. The generation of ADO and IL-10 by these cells allows them to grow in vitro through autocrine signaling that is mediated by ADORA1 and A2A. Morandi F., 2018]. Ecto-5'nucleotidase

regulates the production of extracellular adenosine. Ectonucleoside triphosphate diphosphohydrolase-1 (CD39), which converts ATP to AMP, and CD73, which converts AMP to extracellular adenosine, hydrolyze ATP stepwise in response to tissue damage, inflammation, and hypoxic stress. ATP is released from stressed, necrotic, and/or apoptotic cells during these conditions. Inflammation is increased when ATP receptors are activated, while inflammation is reduced when ATP is broken down to extracellular adenosine and adenosine receptors are activated. A1R, A2AR, A2BR, and A3R are the four adenosine receptors that receive signals from extracellular adenosine. Early research on the anti-inflammatory effects of methotrexate and groundbreaking work showing that A2AR signaling is crucial for preventing tissue-destructive inflammation are among the studies that first connected extracellular adenosine to immunosuppression. 2020 [Harvey J.B.]

Adenosinergic ectoenzymes are present in populations with regulatory functions, such as myeloid-derived suppressor cells (MDSC) and mesenchymal stem cells (MSC). According to a recent study, non-small cell lung cancer (NSCLC) patients' tumor tissues and peripheral blood contain CD11b+CD33+ MDSC that also express the surface ectonucleotidase CD73. Such expression is connected to MDSC's immunosuppressive and chemoprotective properties, which promote the development of NSCLC. On our end, we demonstrated that MDSC isolated from patients with multiple myeloma's bone marrow (BM) niche express CD73, which aids in the local synthesis of ADO. The antitumor immune response is predicted to be compromised by ADO in the bone marrow. The presence of CD73 in MSC was confirmed by additional reports. When there is a high concentration of ATP, as occurs in tissue damage, these cells may result in the production of ADO. However, active T cells are required for this to happen. F. Morandi (2018). There is mounting evidence that adenosine is preferentially produced in the intestinal microenvironment. The majority of CD4+ T lymphocytes in the LP and among IEL, including Foxp3 Tconvs, display CD39 and CD73, the enzymes that successively dephosphosphorylate ATP to generate adenosine, as we specifically demonstrate here. These findings complete earlier research that indicated animals missing CD73 had higher colonic inflammation. RA is not necessary for T-regs to express CD73. The population of CD103+ dendritic cells in the LP, the MLN dendritic cells, and the stromal cells that express aldh1a2, a retinal dehydrogenase involved in the conversion of retinal into RA, are likely related to the source of RA. TGF- and RA are hence cofactors of T-reg formation in addition to favoring Tconvs' ability to produce adenosine, enhancing the immunosuppressive effect. Our findings strongly imply that Tconvs possess higher CD73 levels since TGF- and RA-activated Th1 cells in vitro or in vivo raised CD73. Adenosine must be created constitutively in the gut milieu based on the fact that the majority of intestinal CD4+ T cells can break down extracellular ATP produced at high levels by gut flora. In 2015, Oldenhove G. et al.

Overexpression of CD73 is common in human malignancies, and it is linked to a poor prognosis. Drug resistance, the epithelial-to-mesenchymal transition (EMT), cancer cell proliferation, and cancer stemness are all associated with CD73. In mice lacking the A2BR gene and mice given A2BR antagonist treatment, tumor growth is also slowed. On various different types of immune cells, as listed below, activation of A2AR and to a lesser extent A2BR favors immunosuppression [Harvey J.B., 2020]. Other studies have shown that cancer-derived MSC express functional CD73, making them capable of producing ADO and suppressing T cell activities as a result. F. Morandi (2018). Both CD73 and A2AR inhibition are efficient in reducing tumor growth and metastasis when used alone. However, the effectiveness of combining these treatments in lowering tumor development, the burden of metastatic disease, and extending mouse life is much greater. Increased IFN- production, as well as CD8+ T and NK cell activity, are required for these outcomes. A2AR coupled with anti-PD-1 therapy is most successful, according to research, when cancer cells display high levels of CD73. It is noteworthy that anti-PD-1 therapy is notably synergized by suppressing CD73. According to the latter, CD73 expression may help identify patients who will benefit most from a combination of anti-PD-1 therapy and A2AR inhibition. Although CD73 is a subpar immunotherapy pretreatment biomarker for melanoma, its level of expression in relapse tumors has prognostic significance. As a result, the biomarker CD73 may indicate a malignancy. Additionally, blocking A2AR or CD73 enhances the effectiveness of anti-CTLA-4 therapy in melanoma. Anti-CD73 therapy and 4-1BB agonist therapy recently demonstrated the ability to reestablish anticancer immunity. An activation-induced T cell costimulatory molecule called 4-1BB promotes the activity of cytotoxic T cells and NK cells. Patients with GI cancer are

participating in clinical trials for 4-1BB therapy. In the past, 4-1BB therapy has raised concerns about poor efficacy and toxicity. Additional preclinical research is necessary [Harvey J.B., 2020]. In particular, through A2aR-mediated control of inflammatory processes in several diseases, including autoimmune-mediated damage, adenosine has emerged as a sensor and regulator of tissue injury. Adenosine is also a recognized signaling molecule in the central nervous system (CNS), and changes in its metabolism in tissues reflect changes in metabolic balance. The role of this purine metabolite to chronic autoimmune neuroinflammation is somewhat anticipated in view of the documented effects of adenosine [Ingwersen J. et al, 2016].

Additionally, individuals responding well to adoptive T cell transfer therapy exhibit increased CD73 expression. Therefore, future strategies that combine A2AR inhibition, anti-PD-1 treatment, and/or adoptive T cell transfer with CD73 targeting may be advantageous. Blocking CD39 and CD73 or CD73 and A2AR in head-to-head comparison studies has also shown potential for greatly boosting antitumor immunity. When CD73 and A2AR are co-targeted, the A2AR blockade's compensatory rise in CD73 is prevented. While targeting two independent processes, co-targeting CD39 and CD73 is advantageous. A reduction in CD39 increases ATP levels. High ATP levels enhance the antitumor immunity advantages of CD73 blockade by promoting DC and macrophage antitumor activity. Preclinical research supports the use of CD73 anti-antibodies or small molecule inhibitors in combination with chemotherapy or targeted treatments, such as antibodies against the EGFR. Controlling the growth and spread of melanoma tumors in mice is significantly aided by the use of BRAF and MEK inhibitors in conjunction with A2AR inhibition. The combination of BRAF and MEK inhibitors has the advantage of reducing CD73 expression. As a result, this combination technique has the benefit of reducing CD73 expression without the need for further medication or antibody therapy. Understanding the therapeutic potential of CD73 and/or adenosine receptor inhibition in these malignancies will depend on preclinical research on GI cancers. 2020 [Harvey J.B.]

CHAPTER II: CD73 and its application in multiple pathologies

1. CD73 in Mammalian Physiology and Human Disease

In three families with symptomatic arterial and joint calcifications, work from the National Institutes of Health Undiagnosed Diseases Program in 2011 discovered NT5E missense mutations leading to catalytically impaired CD73 activity. Because in vivo mice models do not accurately reflect the main symptoms of the human disease, it has been difficult to pinpoint the precise mechanisms by which these mutations contribute to the pathogenesis of arterial calcifications due to CD73 deficiency. In 2019, Minor M. et al.

The fact that humans express a number of transcript variants as a result of alternative splicing is one of the most significant differences between NT5E in humans and other species. Direct evidence exists for the reciprocal control of the longer CD73 (CD73S) polypeptide-encoding NT5E-2 transcript and the normal NT5E-1 transcript. Most human tissues express NT5E-2 at low levels at rest, while liver cirrhosis and cancer have higher levels of NT5E-2 and CD73S expression. The COOH-terminal catalytic/dimerization domain of CD73S is 50 amino acids shorter than that of canonical CD73, which impairs dimerization and enzymatic activity. Additionally, in vitro overexpressed CD73S engages in interactions with canonical CD73 and facilitates its proteasomal degradation, operating in a dominant-negative manner. Future studies must incorporate results from in vivo research using Nt5e mice with models obtained from humans due to the species variations in CD73 regulation and related disease characteristics. In 2019, Minor M. et al.

2. Functions in the Lymphatic system: HEMATOLOGY and IMMUNOLOGY

1. ROLE of CD73 IN HEMATOLOGY

Through the coordinated activity of the ectoenzyme CD73, extracellular adenosine (ADO) is produced from ATP or ADP and causes autocrine and paracrine actions that are mediated by type 1 purinergic receptors. The expression of CD73 by chronic lymphocytic leukemia (CLL) cells has been tested to see if it activates an adenosinergic axis that affects growth and survival. Immunohistochemistry demonstrates that CD73 is only found in proliferative hotspots. The highest levels of CD73 expression are found on Ki-67+ CLL cells, close to T lymphocytes, and are also found in perivascular regions. ADP is converted into ADO by CD73+ CLL cells in a time- and concentration-dependent manner. 97/299 (32%) of CLL patients have peripheral blood CD73 expression, which co-localizes with ZAP-70 and CD38 expression. Type 1 purinergic A2A receptors, which are constitutively expressed by CLL cells and are further increased in growing neoplastic cells, are activated by extracellular ADO produced by CD73. ADO receptor activation raises cytoplasmic cAMP levels, decreasing chemotaxis and reducing drug-induced spontaneous death of CLL cells. These findings support the engraftment of leukemic cells in growth-favorable niches while also shielding them from the effects of chemotherapeutic drugs, which is compatible with the establishment of an autocrine adenosinergic loop [Serra S. et al, 2011]. An evolutionary conserved system known as the purinergic signaling pathway controls immunological homeostasis by converting extracellular ATP to extracellular adenosine through successive breakdown via CD73. Adenosine is either produced from extracellular adenine nucleotides or released from stressed or wounded cells as a result of CD39 and CD73 working together. According to Jendrossek V. (2019), CD39 catalyzes the breakdown of ATP and ADP to AMP, whereas CD73 transforms AMP to adenosine.

Tradition has it that immunely compromised monoclonal B cells expressing CD5 and CD23 are the cause of chronic lymphocytic leukemia (CLL), which develops over time. However, recent research has shown that up to 1% of the leukemic clone renovates everyday, suggesting that in patients with indolent clinical illness, a sizable portion of neoplastic cells must die each day. The current, most widely accepted theory attributes CLL's cellular turnover to specific lymph node (LN) and bone marrow (BM) niches. Here, Ki-67+ proliferating CLL cells that interact with CD4+ T cells and the diverse stromal components are found in anatomically defined formations known as proliferation centers. Studies utilizing autologous blood serum or conditioned medium of stromal cells [Serra S. et al, 2011] suggest that soluble mediators in addition to cell-cell interactions also promote CLL survival and proliferation.

In mice lacking CD73, the essential function of CD73 in vascular permeability is clearly seen. Prior research has shown that lymphocyte homing is normal in unchallenged conditions, but in inflammatory and hypoxic conditions, there is an increase in endothelial leakiness and leukocyte extravasation via high endothelial venules (HEV), and the acute immune and inflammatory responses are also exacerbated. Previous histochemical tests using the substrate 5'-AMP showed that human lymphatic vessels express 5'-nucleotidase activity in large amounts. The molecular makeup, functional significance, and differences between this lymphatic (ecto)enzyme and the well-studied blood vessel ecto-5'-nucleotidase/CD73 remain unknown. Using mouse and human systems, it has been shown that lymphatic endothelium expresses CD73 differently from blood vessel endothelium in terms of its functional properties. Along with notable variations in ATP metabolism between lymphatic and blood vessel endothelium generally, it was also shown that lymphatics are markedly heterogeneous and that there are notable species-specific variations in CD73 expression on lymphatics [Algars A. et al., 2011]. The tumor microenvironment is thought to be characterized by an enhanced turnover of extracellular nucleotides and nucleosides as well as by an up-regulation of the ectoenzymes that break them down. Increased CD73 expression and activity have been observed in a variety of solid tumors and leukemia subtypes, suggesting that these factors may favor tumor cell survival and facilitate the spread of metastatic disease. Multiple pathways, including ADO-mediated autocrine and paracrine mechanisms, may be used to produce these effects. The functional significance of autocrine ADO synthesis supporting the survival of leukemic cells and their expansion is demonstrated by the expression of CD73 by CLL cells [Serra S. et al, 2011].

In the blood or the LNs, CLL cells behave very differently and virtually exclusively proliferate in the latter. ADO production may be localized to particular tissue niches and to particular cell subpopulations therein, as shown by the fact that CD73 is primarily restricted to CLL proliferation foci and restricted to cells with a prolymphocyte and paraimmunoblast shape. The CD38 and ZAP-70 gene expression levels are elevated in the CD73+ patient subpopulation, which are both poor prognostic indicators. The CD73+ subset is also more likely to contain Ki-67+ (proliferating) CLL cells, indicating that it has a higher CLL cell turnover rate [Serra S. et al, 2011]. In perivascular regions, CLL cells have high levels of CD73 expression, which may indicate that the molecule is involved in chemotaxis and homing activities. According to models that contend that the adenosinergic axis controls immune cells' motility, CD73-generated ADO inhibits migration by activating the A2A receptor [Serra S. et al, 2011]. Leukocyte entry to infection sites is necessary for pathogen eradication, and continuous lymphocyte cycling between blood and lymphoid tissues is essential for immune system function. Leukocyte trafficking depends on proper contact between leukocytes and endothelial cells in lymphatic and blood arteries. Although the endothelium of blood vessels and lymphatic tissues exhibit many similarities, including the expression of some similar molecules, they also differ noticeably in terms of their appearance, purpose, and chemical makeup [Algars A. et al, 2011].

The role of CD73 in the development of ADO has been demonstrated in a number of experimental models, and this work supports those findings. Purified CLL cells generate extracellular ADO at levels consistent with the range of concentrations found in tumor tissues. ADO production is inhibited when CD73 biochemical activity is blocked by a particular inhibitor, demonstrating that CD73 is the primary surface ectoenzyme producing ADO in CLL. The tissue distribution and enzymatic data taken into account collectively point to [Serra S. et al, 2011]:

The rate-limiting enzyme CD73 plays a role in the activation of the adenosinergic axis. CD73 expression, which is severely constrained in its ability to produce ADO, is closely controlled by environmental signals.

The proliferation hubs of the CLL LNs are where most ADO is produced. It has several anti-inflammatory properties that are mediated by four distinct type 1 purinergic receptors. A2A receptor expression is constitutively high in CLL cells and is even higher in leukemic cells that are actively growing. A2A initiates a signaling pathway that raises cytoplasmic cAMP levels, which modulates other signaling pathways. Resting CLL cells treated in vitro with a selective agonist showed evidence of A2A activation, and this activation was found to increase during proliferative responses. These results show that the adenosinergic axis is primarily active in tissue niches where extracellular nucleotides are higher due to increased cellular turnover, the enzymatic machinery is expressed, and the downstream receptors are abundant. According to multicolor confocal imaging, growing CLL cells that exhibit favored interactions with nearby nonneoplastic T cells are often associated with CD73 expression [Serra S. et al, 2011]. By converting extracellular AMP to adenosine and controlling the permeability of blood arteries and leukocyte flow into tissues, CD73 participates in the extracellular ATP metabolism. Additionally, it can be found on lymphatic vessels, however its distribution and function are still unknown. Contrary to LYVE-1 and podoplanin, which are found on both types of lymphatics, CD73 is absent from efferent lymphatics but is expressed on a subpopulation of afferent lymph vessels. Since lymphatic endothelium has higher ecto-5'-nucleotidase/CD73 activity and lower NTPDase activity than blood vessel endothelium, its extracellular nucleotide metabolism is different from that of blood vessel endothelium. While CD73-deficient lymph arteries mediate lymphocyte trafficking as efficiently as the wild-type lymphatics, the absence of CD73 on lymphocytes reduces lymphocyte migration to the draining lymph nodes by more than 50% in knockout mice. Therefore, despite the fact that endothelial CD73 is crucial for blood vessel permeability and leukocyte extravasation, it does not play a part in these processes on lymphatics. Instead, according to Algars A. et al. (2011), lymphocyte CD73 plays a crucial role in lymphocyte migration through afferent lymphatic arteries.

It is debatable how ADO affects apoptosis. While some research indicate that it decreases viability, stops the cell cycle, and triggers apoptosis, others argue that it plays a cytoprotective effect. The expression of several ADO receptor types, which may activate or prevent the creation of intracellular cAMP and by the ensuing relative increase or reduction in cAMP concentrations, is a potential explanation for these conflicting observations [Serra S. et al, 2011]. The only molecule on the surface of the lymphocyte known to be necessary for trafficking via this pathway is CCR7, despite the fact that lymphocyte migration via afferent lymphatics into the draining lymph nodes is a crucial component in an appropriate immune response. A chemokine receptor called CCR7 binds to the CCL21 chemokine that is shown on the surface of lymphatics. As a result, finding that CD73 is involved in this process gives lymphocyte migration within afferent lymphatics new dimensions and raises the possibility that cell trafficking via the lymphatics is also a complex phenomenon involving structurally different molecular families, as is the case with lymphocyte extravasation from the blood stream into the tissues [Algars A. et al, 2011]. Extracellular ADO contains strong anti-apoptotic properties that can effectively shield CLL cells from natural or etoposide-induced death. These effects are visible throughout a narrow dose range (30–60 M), are consistent with the quantities found in HPLC experiments, and are consistent with the evidence that points to an ADO impact that is dose-specific. ADO works via activating the A2A receptor, as shown by the discovery that an A2A receptor agonist has similar effects [Serra S. et al, 2011].

Together, these findings imply that inhibiting the adenosinergic axis may have significant therapeutic benefits for slowing the course of CLL and/or amplifying the effects of chemotherapy. This objective might be accomplished by inhibiting CD73, a strategy that has been suggested for solid tumors. However, given that soluble CD73 appears to be one of the primary phosphatases that convert fludarabine phosphate (Fludara) into the active medication, this would be problematic in the setting of treatment for CLL. Although other serum phosphatases might make up for the absence of CD73, patients getting fludarabine-based chemotherapy should take this particular concern into account. Alternately, it may be possible to foresee the use of A2A receptor antagonists to prevent the rise in cytoplasmic cAMP levels brought on by antiapoptosis and chemoresistance.

There are multiple distinct A2A receptor antagonists, and Preladenant has undergone Parkinson's disease clinical trials [Serra S. et al., 2011]. Traditional theories linking the blood vasculature's CD73 to permeability were eventually supported by research using CD73-deficient mice. Although CD73 expression is higher in lymphatic endothelium than in blood vessel endothelium, this may not be related to the endothelial barrier function because lymphatic vessels are discontinuous and lack tight junctions in a similar way to blood vessels, making them naturally leaky. Surprisingly, we were unable to identify a function for endothelial CD73 on lymphatics in promoting leukocyte migration and trafficking. Contrarily, CD73 expressed on lymphocytes appears to be crucially engaged in the movement of lymphocytes through afferent lymphatics. The cell type and its chemical composition play a crucial role in the function and behavior of CD73 [Algars A. et al, 2011].

Adenosine triphosphate (ATP) and adenosine (ADO), respectively, are extracellular nucleotides and nucleosides that may contribute to the development of circumstances that favor tumor growth and survival while inhibiting host immune responses. Multiple type-2 purinergic and pyrimidinergic (P2Y and P2X) receptors are bound by extracellular ATP, which affects cellular metabolism, migration, proliferation, and death. Ectonucleotidases, which are surface molecules having catalytic sites in the extracellular compartment, may also use nucleotides as substrates. For instance, CD39 hydrolyzes ATP or ADP to AMP, and subsequently CD73, either soluble or membrane-bound, quickly degrades AMP to ADO. When ADO interacts with a particular class of type 1 purinergic G protein-coupled receptors, it can either be taken up by cells to replenish the nucleotide pool or it can trigger powerful immunosuppressive and anti-inflammatory reactions (A1, A2A, A2B, and A3). Producing ADO is a crucial part of regulatory T cells' suppressive machinery because it inhibits the proliferation of effector T cells and the release of T-helper 1-type cytokines [Serra S. et al., 2011]. Only lymphocytes can exit the lymph nodes via the efferent lymphatics, despite the fact that several leukocyte subtypes can enter the draining lymph nodes via the afferent lymphatics. Since none of the currently recognized markers used to identify lymphatics can distinguish between afferent and efferent lymphatics, no biological foundation for this phenomena have been discovered. Therefore, CD73 stands out among the lymphatic markers due to its preferential expression on afferent lymphatics but not on efferent lymphatics. Based on this pattern of expression, it is possible to hypothesize that, even though CD73 on afferent lymphatics does not appear to directly contribute to the leukocyte trafficking, its presence on afferent lymphatics alters the adenosine milieu in afferent and efferent lymphatics and may, under certain circumstances, indirectly contribute to cell migration in these various arms of lymphatics. Certain lymphocyte subpopulations, including T-reg, regulatory B cells (B-reg), and endothelial cells, express CD73 on their surface, which is crucial to their regulatory roles. However, stromal cells, mesenchymal stem cells (MSCs), and tumor-associated stem cells also express CD73. Pre-clinical research has shown that CD73 on stromal cells and tumor cells plays a role in the homing and stemness of cancer stem cells, as well as in the inhibition of immune-mediated responses. Furthermore, MSCs were able to upregulate T cell CD73 expression, which further supported their immunosuppressive role. In a pre-clinical model of pancreatic neuroendocrine tumors, CD73 inhibition inhibited tumor growth and cancer stem cells' capacity to metastasize. Therefore, stem cell-mediated immunosuppressive or regenerative processes may facilitate cancer cell escape from either anti-cancer immunotherapies or natural anti-tumor immune responses. Adenosine triggers the expansion or differentiation of myeloid-derived suppressor cells (MDSC), M2-like macrophages, as well as T-reg and B-reg, as well as the suppression of inflammatory functions of cells from the innate and adaptive immune systems. This contributes to the development of regulatory environments. Additionally, naive T-cell homeostasis, memory cell survival, and maybe T cell development may all be impacted by CD39/CD73-dependent production of adenosine [Jendrossek V., 2019].

Adenosine has strong anti-inflammatory properties and affects endothelium and leukocyte sites. When the adenosine receptors A2A and A2B are activated, L-selectin shedding is inhibited, up-regulation of 2 integrins is reduced, and leukocyte adherence to the microvascular endothelium is reduced. Adenosine also lessens leukocyte extravasation and immunological responses by reducing cytokine production from the vasculature and leukocytes. As the absence of CD73 from lymphatic endothelium did not affect lymphocyte trafficking via the afferent lymphatics, it is clear that these mechanisms are not assisting in the normal migration of lymphocytes

from the periphery to the draining lymph nodes. In this context, it's important to remember that just a tiny fraction of the mouse skin's afferent lymphatics is CD73 positive. Humans, on the other hand, have a condition where the majority of afferent lymphatics express CD73. In order to determine if CD73 plays a substantial role in leukocyte trafficking under this inflammatory challenge, we administered LPS to the skin and challenged it. Intriguingly, LPS injection caused a more prominent macroscopic inflammation in CD73-deficient mice than in wt mice, which is consistent with the CD73-deficient mice's blood vessel leakiness at inflammation locations [Algars A. et al, 2011].

2. ROLE of CD73 IN IMMUNOLOGY

1. CD73 Immune Checkpoint Pathway

Immune checkpoint therapy-related tumor development may be linked to deficiencies in tumor antigen presentation, which prevent T cells from recognizing tumor cells. Natural killer (NK) cell activation-based medicines have been created as a result of this. Granular lymphocytes with the ability to produce large amounts of inflammatory cytokines and trigger cytotoxic reactions against virus-infected and altered cells are known as NK cells. In addition to complex mechanisms and a large repertoire of signals from both activating and inhibiting receptors, the microenvironment's cytokines and neighboring cells play a role in how these cells' functions are regulated. Despite the fact that NK cells enter malignancies infrequently, it has been demonstrated that their presence in tumor biopsies is favorably correlated with improved survival [Yong Neo S. et al, 2019]. The blocking of numerous immunological checkpoints is one of the most promising methods for triggering therapeutic antitumor immunity. Immune checkpoints are several inhibitory pathways that are built into the immune system and essential for preserving self-tolerance. They also regulate the length and intensity of physiological immune responses in peripheral tissues to reduce collateral tissue damage.

In the tumor microenvironment, the cell surface enzyme CD73 (cluster of differentiation 73), also known as 5'-nucleotidase (5'-NT) or ecto-5'-nucleotidase, is overexpressed (see Table 2) and aids in the formation of the tumor by reducing anti-tumor immunity through the adenosine receptor pathway [Kobie JJ, 2006]. It is an enzyme that frequently does the AMP to adenosine conversion. [Antonioli, L.; et al., 2013] CD73 is made up of a dimer of two identical 70-kD subunits that are joined to the exterior face of the plasma membrane by a glycosyl phosphatidyl inositol linkage. This enzyme, known as ecto-5’-nucleotidase, helps lymphocytes attach to endothelial cells and changes extracellular AMP into the strong anti-inflammatory compound adenosine. However, little is known about the control of CD73's expression and function. On endothelial cells, IFN- causes a long-lasting up-regulation of CD73 at the protein and RNA levels, but not on lymphocytes. Additionally, endothelial cells treated with IFN- have enhanced CD73-mediated adenosine synthesis, which lowers their permeability. No discernible changes in the level of CD73 expression on endothelial cells are seen after stimulation with PMA, FMLP, dibutyryl cAMP, thrombin, histamine, IL-1, TNF-, and LPS. After IFN- was administered intravenously to treat urinary bladder tumors, CD73 was found to be up-regulated on the vasculature in vivo [Niemelä j. et al., 2004]. High levels of adenosine may reduce the ability of natural killer (NK) cells to produce tumor necrosis factor (TNF) and interferon (IFN). Additionally, adenosine may inhibit NK cells' lytic activity in a way that is dependent on the A2A receptor, which would limit their ability to mediate the lysis of tumor cells. Adenosine additionally had an impact on macrophages. Adenosine's interaction to the A2A receptor on macrophages caused the production of IL-4 and IL-10, which helped to suppress the immune system's response to the tumor and aided tumor growth (Zhang H-Z. et al., 2014).

As the enzyme that really breaks down AMP into adenosine in the last stage of the degradation process, CD73 plays a crucial role in inducing immunosuppression by breaking down adenosine triphosphate (ATP) into adenosine. ATP quickens the tumor microenvironment's immune cells' ability to kill tumor cells. Adenosine

buildup, on the other hand, promotes angiogenesis, immunological suppression, and dysregulation of immune cell infiltrates, all of which contribute to the spread of tumors [Antonioli, L.; et al., 2013]. Adenosine can be produced and released into the tumor microenvironment by CD73, which lowers immunological function. CD73 inhibition might increase T-cell activity;

- ☐ The regulatory T cells' cell surface enzyme is called CD73 (T-regs)

- ☐ Immunological-activating ATP must be converted by CD73 into immunosuppressive adenosine, whose release aids T-regs in inhibiting immune activation.

Adenosine is produced when CD73 transforms AMP into adenosine, which may have immunosuppressive effects on T cells. CD39 catalyzes the conversion of ATP or ADP into AMP. [JJ Kobie, 2006]

Always overexpressed in various cancer types is CD73 (see Table 2). The expression of CD37 has been linked to a pro-metastatic phenotype in prostate cancer and a poor prognosis in melanoma, colorectal, gastric, and triple negative breast cancer [Antonioli, L.; et al., 2013]. Cancer uses CD73's ability to lower antitumor immunity to its advantage. Tumor cells express CD73 and release adenosine into the tumor microenvironment in a manner akin to T-regs [Kobie JJ, 2006]. Breast malignancies are epithelial tumors, whereas sarcomas are mesenchymal tumors, hence their effects on NK cell infiltration and activity may vary. Although NK cell gene signature and tumor NT5E expression showed a favorable connection, CD73 was also discovered to be expressed on tumor-infiltrating NK cells. According to Yong Neo S. et al. (2019), the proportion of CD73+ tumor-infiltrating NK cells also positively linked with bigger breast cancers. IFNs are powerful immunomodulatory agents. They inhibit viral replication, cause antiviral resistance, and control immunological reactions. IFN- levels are typically modest in healthy people, whereas APCs release IFN- from the start of inflammation in inflammatory diseases. The lower amounts employed in our tests that induce CD73 to up-regulate are comparable to those naturally present in patients with inflammations, and are thus anticipated to cause CD73 to up-regulate also in vivo. IFN- has been demonstrated to improve the endothelial barrier function of bovine retinal microvascular endothelial cells, which is intriguing since it suggests that IFN- maintains the integrity of the vascular wall. Although Gillies and Su's earlier study did not fully explain the reasons, our current findings strongly imply that up-regulation of CD73 contributes to this occurrence by increasing adenosine synthesis [Niemelä j. et al., 2004]. A critical homeostatic equilibrium of extracellular adenosine levels is orchestrated by the CD73 metabolic immune checkpoint as part of a negative feedback system to regulate inflammatory responses in a stressed or injured tissue milieu. The absence of CD73 expression may indicate normal tissue microenvironment immunomodulation and wound healing. However, as a tumor progresses, metabolic stress builds up inside the tumor microenvironment (TME), which results in dysregulated CD73 expression and activity in malignancies such breast cancer, metastatic melanoma, and ovarian cancer. Due to adenosine synthesis dysregulation, overexpression of CD73 in a tumor also aids in metastasis, anthracycline resistance, and immunological evasion. Due to these factors, CD73 inhibitors are currently utilized in combination with other cancer treatments, such as anti-PD-1/anti-PD-L1 therapy, in cancer immunotherapy [Yong Neo S. et al., 2019]. IFN- has diverse impacts on CD73 on endothelial and lymphocyte cells, demonstrating the different ways that different cell types regulate CD73 expression. Since both B and T cells express high affinity IFN-receptors, the lack of IFN-receptors on lymphocytes cannot account for this discrepancy. Although the cDNA sequences of CD73 in different cell types are almost identical, triggering CD73 with an anti-CD73 mAb (which mimics the ligand binding) leads in the shedding of lymphocyte CD73 but not endothelial cell CD73. It is important to note in this context that CD73 levels in lymphocytes and endothelial cells differ significantly. Only 10–15 percent of lymphocytes express CD73; this is a low expression rate as compared to, say, HUVEC, which all express CD73. The optimal behavior of lymphocytes, whose function is to actively deaminate the pre-existing adenosine and extravasate to lymphoid tissues or sites of inflammation, may depend on these types of cell-specific changes in CD73 quantity and regulation. In contrast, endothelial cells require adenosine in order to continue functioning as a barrier [Niemelä j. et al, 2004]. Similarly, research demonstrated that NK cells gain the expression of CD73 after physical contact with human umbilical cord–derived MSCs or dental pulp stem cells. It has been discovered

that NK cells from gastrointestinal stromal tumors (GISTs) exhibit more surface CD73 in malignancy. However, the resilience in phenotype and function of tumor-infiltrating NK cells is not yet well understood. Here, we expand on these findings and show that NK cells upregulate the expression of CD73 as well as a number of other immunological checkpoint receptors that are linked to immune exhaustion. To gain noncanonical roles to reduce the immunological environment, NK cells underwent transcriptional reprogramming [Yong Neo S. et al, 2019].

By influencing several types of immune cells, CD73 and other adenosinergic molecules are essential in the development of an immunosuppressive TME. The functions of CD73 on the major immune cell types are next briefly discussed [Zhang B. et al, 2020].

Table 2 Summary of key ecto-5'-nucleotidase (CD73) applications in experimental environment involving specific targets cells and associated enzymes, proteins, genes or receptors.

Cell	Enzyme/Protein/ Gene/Receptors	Applications
Regulatory T (T-reg) cell	CD73 / A2AR	☐ Although CD73 is expressed on many T cell subsets in mice, Foxp3+ T-regs have the highest levels of this molecule. ☐ CD73 is essential for T-reg-mediated regulation of effector T cell function, as evidenced by T-reg cells' decreased immunosuppressive capacity in mice with tumors that lack CD73. ☐ A2AR on T effector cells primarily mediates these effects. ☐ In humans, CD73 expression in T-regs is low, but it is elevated in some cancer patients, particularly in those who have had high-dose IL-2 therapy for melanoma. ☐ CD73 inhibition lessens T-reg-mediated immunosuppressive activity, similar to mouse cell system
Effector T cell	CD73/ CD8+ / A2AR	☐ A high amount of CD73 is linked to a worn-out or lethargic phenotype of T cells. ☐ Th17 cells express CD73, which inhibits the function of effector T cells that depend on CD73's enzymatic activity. ☐ By boosting Th17 cells' effector activity, genetically eliminating CD73 or lowering CD73 by reprogramming them increases anticancer effects. ☐ As predicted, treatment with an A2AR agonist reduces T cell growth and activation and increases anergy. ☐ A recent study validated the prognostic usefulness of CD8+ T cells expressing CD73, especially following immunotherapy, despite the fact that the function of CD73 by effector CD8+ T cells is still unclear.
Natural killer (NK) cell	CD73 / A2AR	☐ NK cells exhibit low levels of CD73 expression, which can rise under certain circumstances. ☐ Tumor-infiltrating NK cells express more CD73 than PBMCs do in gastrointestinal stromal tumors.

Cell	Enzyme/Protein/ Gene/Receptors	Applications
		☐ CD73 was also discovered on NK cells isolated from mouse melanoma, indicating that CD73 expression may develop on tumor-infiltrating NK cells. ☐ A2AR is the main receptor via which CD73-produced adenosine reduces NK cell activity. ☐ Activation of the A2AR prevents the development, activation, and cytotoxic activity of NK cells. ☐ On the other hand, NK cell lack of A2AR signaling reduces CD73+ tumor spread and boosts anti-tumor immune response. ☐ By adopting CD73 in the TME, NK cells underwent a phenotypic and functional flip to an immunosuppressive population, highlighting the significance of CD73 targeting for NK-based immunotherapy.
Myeloid derived suppressor cell (MDSC)	CD73/CD39 / A2BR/ TGF-β	☐ Patients with cancer have greater CD73 levels on MDSC. ☐ A2BR antagonist decreased the increase of tumor-infiltrating MDSCs in TME and this resulted in the delayed tumor growth in a mouse model. CD73-mediated adenosine boosts MDSC function primarily through A2BR. ☐ CD73 on MDSCs was increased by tumor-derived TGF- via the mTOR/HIF-1 pathway ☐ CD39+CD73+ In the NSCLC patients, MDSCs represented an unique inflammatory subpopulation linked to immunosuppressive markers and chemotherapeutic response. ☐ It was discovered that metformin decreased CD73 through activating AMP-activated protein kinase, preventing MDSC activity in ovarian cancer patients. ☐ Inhibiting MDSCs while targeting CD73 enhances antitumor immunity in part.
Macrophages	CD73 / CD39 / $CD4^+$	☐ CD73 is expressed on resident macrophages, and its amount of expression varies according to the macrophages' level of activity. ☐ By transitioning between the M1 and M2 phenotype, macrophage function is determined by modulating CD73 activity. ☐ Tumor-associated macrophages (TAMs) produce CD39/CD73, which inhibits the proliferation of CD4+ T cells by generating adenosine. ☐ Reduced M2 polarization of TAMs with less CD73 and lower adenosine levels in the TME were associated with fasting-mediated tumor inhibition.

Cell	Enzyme/Protein/ Gene/Receptors	Applications
		☐ The fine-tuning of TAM function during tumor growth requires CD73 and CD39 activity.
B cell	CD73 / APCP/ IL17A /IgG2b	☐ The majority of B cells in adult humans express CD73, but neonatal B cells do not, and this lack appears to be the cause of reduced B cell activity in early life. Additionally, CD73 is necessary for class switch recombination in B cells, which is thought to be a sign of B cell maturity. ☐ CD73 activity in B cells was found to be crucial for tumor formation in a mouse melanoma model. ☐ Adenosine 5'-(,-methylene) diphosphate (APCP), a CD73-specific inhibitor, treatment increased IL17A and made it easier for B cells to exist and produce IgG2b in the melanoma.

One of the characteristics of cancer has long been thought to be immunoescape. Tumor cells use a variety of strategies to avoid immune monitoring during the growth, progression, and metastasis of a tumor. Adenosine signaling is a component of one such pathway. CD73 is a crucial molecule in tumor immunoescape because it promoted the breakdown of AMP into adenosine as part of the purinergic signaling cascade. Adenosine produced by CD73 can bind to four different G-protein-coupled receptors: A1, A2A, A2B, and A3 to mediate its immunosuppressive action. According to Zhang H-Z et al. (2014), adenosine can affect the immune system through a number of different mechanisms.

2. Anti-CD73 in cancer immunotherapy

The principles underpinning tumor biology and immunology have become better understood in recent years, which has led to considerable advancements in cancer immunotherapy. In this regard, CD73 is a crucial molecule because it supports the development of an immunosuppressed and pro-angiogenic niche inside the tumor microenvironment that aids in the start and spread of cancer [Antonioli L. et al., 2016]. An immune checkpoint is created by CD73, an ecto-5'-nucleotidase (NT5E), which produces adenosine (ADO), which inhibits immunological activation via the A2A receptor. Poor clinical outcomes are correlated with elevated CD73 levels in tumor tissues [Yu M. et al., 2020]. Clinical results for some cancer patients treated with immune checkpoint inhibitors that target the PD-1/PD-L1 or CTLA-4 pathway to release tumor-mediated immunosuppression were noticeably improved. Another previously unidentified, non-redundant immunological checkpoint is represented by the greatly enhanced CD73 activity in CAFs through the conversion of eATP to eADO and through the ADO-CD73 pathways in CAF-network [Yu M. et al, 2020].

Immunotherapy chances are significantly impacted by CD73's control of immunity in cancer. Adenosine synthesis rises in cancer due to elevation of CD73 expression in tumor cells and cells in the tumor stroma [Kobie JJ, 2006], which:

- ☐ Suppresses the synthesis of cytokines and the proliferation of T and NK cells, as well as the activity of antigen-presenting cells (APCs).
- ☐ Encourages the proliferation and suppressive activity of regulatory T cells (T-reg).
- ☐ Promotes macrophage M2 polarization and MDSC stimulation.

These adjustments promote disease development and tumor growth.

Inhibiting CD73 activity may improve anti-tumor immune surveillance at the level of T cells and other immune cells regulated by adenosine since preclinical research has linked CD73 to immunological escape in cancer. A crucial tactic to aid in triggering an anti-tumor immune response may be to target the CD73 pathway in conjunction with other possibly complimentary immunological pathways [Kobie JJ, 2006]. In pre-clinical animals, targeting CD73 has positive anticancer effects, and CD73 blockade treatments in combination with other immune-modulating drugs (such as anti-CTLA-4 mAb or anti-PD1 mAb) are particularly appealing. Through the creation of CD73 monoclonal antibodies, anti-CD73 therapy has the potential to be a brand-new biologic treatment for cancer patients [Yu M. et al, 2020]. Multiple malignancies have been treated with IFN-. In this study, we discovered that IFN- treatment particularly increased the expression of CD73 on endothelial cells in bladder cancer patients. The malignant cells of two tumors positive for CD73 or the normal lymphocytes that are always present in varying numbers inside tumor tissues did not exhibit this type of up-regulation. When patients are treated with antimetabolites that prevent the de novo production of purines, CD73 positivity is hypothesized to give the patients a survival advantage as a salvage pathway for CD73-positive tumor cells. While CD73-negative cancer cells have a faster rate of proliferation and are therefore more susceptible to cytotoxic treatments, increased adenosine synthesis in tumors may give normal cells, particularly endothelial cells and CD73-positive tumor-infiltrating lymphocytes, an edge to survive. Because angiogenesis is a requirement for tumor growth, the trophic activities of adenosine on endothelial cells may be helpful to cancer growth as a whole [Niemelä j. et al., 2004]. In collaboration with ecto-nucleoside ATPases, CD73, an ecto-5'-nucleotidase (NT5E), produces extracellular adenosine (eADO). Immune suppression is imposed by extracellular ADO through the adenosinergic A2A receptor. Therefore, by directing the eATP-triggered immune activation signal to eADO-induced immunosuppression, CD73, a rate-limiting enzyme of eADO production, functions as a critical metabolic and immunological checkpoint. Clinical evidence demonstrates the critical function of CD73 in tumor progression and links higher CD73 levels in the tumor tissue of numerous cancer types, including breast, ovarian, and colorectal cancers (CRC), to poor patient survival. Clinical trials are currently testing CD73 neutralization therapy, either by alone or in conjunction with an A2A antagonist. However, the critical populations in the tumor microenvironment (TME) that anti-CD73 targets are not well understood [Yu M. et al., 2020].

Numerous studies have shown how important CD73 is in creating this immunosuppressed milieu, which is marked by elevated adenosine levels and encourages the growth and spread of tumors. The release of immunomodulatory factors by tumor and immune cells inside the tumor microenvironment creates an immunosuppressive milieu that promotes tumor growth. The CD39/CD73 complex affects the ability of T-regs and Th17 cells to suppress tumor growth by preventing the activation, clonal expansion, and homing of tumor-specific T cells (especially T helper and cytotoxic T cells), hindering the ability of cytolytic effector T lymphocytes to kill tumor cells, and promoting the transformation of type 1 macrophages into tumor-promoting cells. The predominant CD73 population in human colorectal malignancies (CRCs) and two CD73 mouse tumor models, including a modified CRC, consists of cancer-associated fibroblasts (CAFs). Clinically, higher CD73 activity and a poor prognosis are closely correlated with high CAF abundance in CRC tissues. The ADO-A2B receptor-mediated feedforward circuit that is activated by tumor cell death, which imposes the CD73-checkpoint, is the mechanism by which CAF-CD73 expression is increased. In CAF-rich tumors, concurrent inhibition of A2A and A2B pathways with CD73-neutralization synergistically improves antitumor immunity. Therefore, increasing therapeutic outcomes requires the strategic and efficient targeting of both the A2B-mediated ADO-CAF-CD73 feedforward circuit and the A2A-mediated immune suppression [Yu M. et al, 2020].

Immunosuppression, angiogenesis, mucosal hydration, and ischemia preconditioning are all brought on by adenosine. Adenosine produced by CD73 during pathophysiological conditions guards against tissue deterioration brought on by inflammation, ischemia, and hypoxia. Extracellular ATP and ADP levels are mostly unaltered in CD73 animals despite the absence of extracellular adenosine signaling. Surprisingly, CD73 mice survive, proving that CD73 is not necessary in healthy physiologic settings. Recent research has discovered that CD73 insufficiency, in patients with an autosomal recessive condition brought on by loss-of-function mutations in Cd73, contributes to arterial calcification. Additionally, CD73 knockout (CD73) mice replicated some of the

traits linked to arterial calcification due to CD73 deficiency (ACDC) [Zhang B. et al., 2019]. Although tumor-infiltrating leukocytes (TILs), particularly regulatory T cells (T-regs), and some tumors, such as melanomas and prostate cancers, have been shown to express CD73, experimental model systems using Cd73null mice strongly suggest that host CD73 activity in the non-hematopoietic compartment plays a crucial role in the observed immunosuppression. CD73 activity in cancer-associated fibroblasts (CAFs), which are the predominate non-hematopoietic stromal cells in many cancers, is largely unknown aside from endothelial vasculature [Yu M. et al, 2020]. The activation of four different adenosine receptors (G protein-coupled receptors)—A1R, A2AR, A2BR, and A3R—in the tumor microenvironment has been demonstrated in prior research to have a detrimental effect for extracellular adenosine. Inhibiting T cell receptor signaling and promoting the production of Foxp3+ regulatory T cells are the results of adenosine activation of the A2AR (T-regs). Adenosine activation of A2BR, on the other hand, reduces the vascular endothelium's ability to operate as a barrier and encourages myeloid cells to develop an anti-inflammatory phenotype, which inhibits immune-mediated tumor cell elimination. We and others have demonstrated that CD73 mice more rapidly reject tumors in comparison to wild-type (WT) mice because of improved antitumor immunity and reduced carcinogenesis [Zhang B. et al., 2019]. These findings are consistent with the findings from A2AR and A2BR mice.

High levels of CD73 in the TME of human CRCs are linked to high CAF abundance and immunosuppression, and CAF expression and bioactivity on CAFs are much higher than that of other cellular components inside the TME. When used therapeutically, simultaneous A2A and A2B antagonism, particularly when combined with CD73 neutralization, significantly improves tumor control.

We go on to show that the CAF-rich TME is where the combined effects of andenosinergic antagonism and CD73-neutralization on tumor control are most effective [Yu M. et al, 2020]. A mouse model of ovarian cancer shows that CD73 on CAFs facilitates tumor immune escape, suggesting that the CD73-CAF identity represents a universal characteristic and a shared immunosuppressive mechanism throughout many tumor types and species. In addition, poor clinical outcomes in CRC patients, CAF abundance, higher CD73 levels in the TME, and faster tumor growth in mouse models have all been linked [Yu M. et al, 2020].

Unexpectedly, a recent study found that poorly differentiated advanced-stage endometrial cancer and ovarian high-grade serous carcinoma had lower CD73 expression. Surprisingly, in these tumors, adenosine produced by CD73 suppresses the spread of the disease. Endometrial carcinomas with permanent cell-cell adhesions mediated by A1R appear to require CD73-generated adenosine to maintain a physiological balance that preserves epithelial integrity. Loss of CD73 accelerates the progression of adenosine deaminase endometrial tumor because the epithelial barrier function is crucial for preventing endometrial carcinomas with fewer intervening stromal or inflammatory cells in interconnected malignant glands. This occurrence is totally distinct from what has been seen in other tumor types, and it is most likely the result of the receptor's context-dependent actions in various tumor types. These results offer a unique viewpoint on the function of CD73-generated adenosine in tumorigenesis [Zhang B. et al., 2019]. In addition to offering a molecular explanation for previously reported therapy- or stress-induced CD73 overexpression, A2B-mediated CD73 upregulation in CAFs and mesenchymal-like malignancies also offers useful practical considerations for blocking immunological checkpoint with the least amount of harm. The eADO level can be quickly increased by pathological or therapeutic-related tissue damage or cell death up to 100 M, which strongly stimulates the A2B pathway for CD73 overexpression despite of A2A blockage. Clinical outcomes improve when CD73-neutralization and A2B blockage work together to reduce the CD73-ADO checkpoint that is frequently present in CAF. In terms of therapy, our findings with two mouse tumor models highlight the value of concurrent suppression of the non-redundant repressive pathway of the A2A and A2B-dependent CD73-ADO immune checkpoint for enhancing anticancer immunity [Yu M. et al., 2020].

Recent discussions have focused on the functions of CD73 on tumor and host cells, as well as its prognostic and therapeutic potential in human malignancies. With this study, we hope to present a more comprehensive understanding of how CD73 contributes to immune evasion and tumor development, emphasizing the value of

combination therapies resulting from CD73 targeting, which is presently being researched in early phase clinical studies. Anti-CD73 immunotherapy's drawbacks, difficulties, and potential future research approaches are also discussed [Zhang B. et al., 2019].

3. ROLE of CD73 IN INFLAMMATORY and INFECTIOUS DISEASES

It has been known for more than 20 years that adenosine reduces possibly detrimental effects of neutrophil activation. More recent research has concentrated on pharmacological agonism/antagonism with receptor-selective analogs or native adenosine to target adenosine receptors to reduce tissue destruction in a number of illnesses [Colgan SP, 2006]. The modulation of endothelial CD73 expression and function is mostly unknown at this time. However, there might be certain inducers produced during inflammation that specifically affect endothelial CD73 expression in vivo. An earlier result that CD73 is up-regulated in inflammatory skin suggested this theory. This work was intended to identify the factors responsible for the regulation of CD73 expression as well as ecto-5'-nucleotidase-mediated adenosine production because adenosine, having an anti-inflammatory and cell-protective effect, plays a significant role in controlling the extent and consequences of inflammation [Niemelä j. et al, 2004].

Numerous cytokines and chemokines are produced under inflammatory conditions, which significantly alter the expression and/or activation state of numerous adhesion molecules. At regions of inflammation, particularly in the skin, expression of CD73 is upregulated, but the mediators responsible for this action are still unclear. In this study, we examined a wide range of rapidly and persistently activating mediators and discovered that IFN- is a powerful inducer of CD73 expression. Infection-related patient concentrations of IFN- caused a selective, dose- and time-dependent in vitro up-regulation of CD73 expression on endothelium but not on PBL. More significantly, it increased CD73 expression in bladder cancer patients' tumor vasculature in vivo. Endothelial cells with up-regulated CD73 following IFN- stimulation have improved barrier function because the enzyme produces adenosine from 5'-AMP [Niemelä j. et al., 2004]. It is now possible to better comprehend the metabolic processes that produce extracellular adenosine (i.e., CD39 and CD73) thanks to more recent studies that make use of new tools and insights. The main point of contact between circulating leukocytes and tissue inflammatory signals is the vascular endothelium. In order to coordinate leukocyte trafficking in response to chemotactic stimuli, the endothelium plays a crucial role. Vascular endothelial cells are in a perfect position to coordinate extracellular metabolic activities necessary for endogenous anti-inflammatory responses thanks to this crucial anatomic location [Colgan SP, 2006].

1. CD73 and Autoimmune Diseases

Autoimmune diseases are a diverse set of illnesses that affect many organ systems or particular target organs. They are chronic conditions brought on by the loss of immunological tolerance to self-antigens. Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), type I diabetes (T1DM), juvenile idiopathic arthritis (JIA), autoimmune hepatitis (AIH), and others are common autoimmune disorders. In order to defend the body from infections, tumors, and other diseases, the primary role of the human immune system is to recognize antigens and then remove any that are not self-antigens. In typical circumstances, an alarm signal triggers a variety of cellular reactions with the goal of preventing an excessive inflammatory response and reestablishing immunological homeostasis. The existence of shared characteristics between autoimmune disorders lends credence to the potential implication of a common point between autoimmune diseases [Morandi F. et al, 2018].

- ☐ non-specific autoantibodies (such as antinuclear antibodies and rheumatoid factor), which include signs and symptoms include arthralgia, arthritis, alopecia, weariness, photosensitivity, and Raynaud's phenomenon;
- ☐ High cytokine levels (including TNF, IL-1, IL-6, IL-10, IL-17, etc.);

- ☐ The presence of phagocytic macrophages, neutrophils, self-reactive CD4+ T helper cells, self-reactive CD8+ cytolytic T cells, as well as smaller amounts of natural killer cells, mast cells, and dendritic cells.

Th1, Th17, and Th9 cells are T effector cells that have a role in the pathophysiology of autoimmune disorders. Rheumatoid arthritis, multiple sclerosis, juvenile rheumatoid arthritis, autoimmune uveitis, and diabetic mellitus are five autoimmune illnesses and/or their associated animal models where ADO may have a role [Morandi F. et al, 2018].

An autoimmune disease mostly affecting the joints is rheumatoid arthritis (RA). The fundamental mechanism combines elements from the environment and the genes. TNF- appears to have a significant role in the overexpression of several cytokines and their receptors in the synovium, and it has been suggested as a potential therapeutic target. In fact, therapeutic advantages were seen in RA patients using the anti-TNF- antibody (infliximab), either alone or in combination with methotrexate (MTX). 2018 [Morandi F. et al]

The most well-known autoimmune condition that targets the central nervous system is multiple sclerosis (MS). According to Morandi F. et al. (2018), the activation of immune-inflammatory, oxidative, and nitrosative stress pathways, which are connected to two key phases, monitors the physiology of MS:

- ☐ lesions and the breakdown of the myelin sheath
- ☐ inflammation

The most prevalent childhood chronic rheumatic disease and a major contributor to both short- and long-term disability is juvenile rheumatoid arthritis. Similar to rheumatoid arthritis, the synovial lymphocyte infiltration is thought to be polarized toward a Th1 proinflammatory response. [Morandi F. et al, 2018]

Autoimmune uveitis is an inflammatory, noninfectious condition that affects the vascular layer of the eye. It can impair vision and potentially cause total blindness if treated improperly and without prompt detection. T helper 1 (producing interleukin-2 and interferon-) and T helper 2 cells (producing interleukins 4, 5, and 13) exert both pathogenic and protective roles, whereas T helper 9 (producing interleukins 9 and 10) and T helper 17 cells (producing interleukins 17A, 21, and 22) play exclusively pathogenic roles. These T cell effector phenotypes and their cytokine pathways appear to be implicated in autoimmune uveitis T helper 17 cells have recently been linked to the pathophysiology of the uvea [Morandi F. et al., 2018].

Both type 1 and type 2 diabetes are brought on by immune-mediated breakdown and malfunction of pancreatic beta cells, which ultimately results in poor glucose-stimulated insulin secretion (GSIS) and beta cell death. According to recent research, infiltrating immune cells in pancreatic islets produce localized amounts of diabetogenic cytokines such IFN-, IL-1, and TNF, which cause inflammation and autoimmune recognition of beta cells. 2018 [Morandi F. et al]

The behavior of ADO as the alarm signal in vivo has been proven by a number of lines of evidence. Target cells' ADO receptors are activated by adenosine, which also causes a number of biological reactions before suppressing the immune response. In animal models of inflammation, it has been shown that locally generated ADO can induce the production of anti-inflammatory cytokines (such as IL-10) and block the release of proinflammatory molecules (such as TNF- and nitric oxide). Indeed, numerous data indicate that the localized faulty production of ADO and the partial or complete loss of activity of the adenosinergic pathways may both contribute to the beginning of autoimmune diseases [Morandi F. et al., 2018]. It takes several steps for lymphocytes and endothelial cells to connect. Circulating cells make use of a highly controlled set of adhesion molecules in order to enter the vessel wall and get to the target spot. Inflammation is characterized by increased adherence to endothelium and subsequent transmigration of recirculating leukocytes through the endothelial lining of the arterial wall into the tissue. Additionally, inflammation-related locations are where pro- and anti-inflammatory

cytokines are released to a significant degree. These cytokines are effective regulators of adhesion molecule expression [Niemelä j. et al., 2004].

i. Multiple Sclerosis (MS)

The most prevalent chronic inflammatory illness of the central nervous system (CNS) in Western nations, multiple sclerosis (MS) is characterized by demyelination, neuronal destruction, and glial scarring. Adenosine levels that are elevated have been seen in MS patients' cerebrospinal fluid. A2aR targeting may prove to be a promising MS therapeutic method because it is expressed in both immune and primary CNS cells. It is difficult to predict whether the receptor would give neuroprotective effects whether it is activated or blocked. The A2aR antagonist SCH58261 was demonstrated to protect against the disease in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Genetic ablation of CD73, a protein that produces adenosine, led to a milder EAE course. Follow-up research revealed that deletion of A2aR worsens EAE pathogenesis and that both CNS and immune cell-derived A2aR regulate EAE [Ingwersen J. et al, 2016]. Recent theories suggest a connection between the production of ADO and the clinical prognosis of multiple sclerosis patients (MS). Endothelial cells (ECs) treated with IFN- are able to boost CD73 surface expression in vitro, according to research by Airas et al. After systemic administration of IFN- to MS patients in vivo, the same impact was also seen in ECs, and an increase in soluble serum CD73 was also seen [Morandi F. et al, 2018]. Additionally, a link between a rise in CD73 and the clinical prognosis of MS patients has been found. This conclusion was supported by the finding that IFN- increased the expression of CD73 on astrocytes and EC originating from the blood-brain barrier (BBB). Following this modification, lymphocyte transmigration through BBB-EC was decreased. Using, -methyleneadenosine-5-diphosphate, a particular CD73 inhibitor, which returned the condition to the way it was before, this specificity of action was confirmed. The IFN-treatment was observed to be followed by elevated levels of soluble blood CD73 activity and skin microvascular CD73 expression in the majority of MS patients, according to the same group of authors. ADO might help explain why IFN- has a positive impact on MS patients. Through the enzymatic activity of CD39 and CD73, the suppression of Th17 cells was connected to the generation of ADO [Morandi F. et al., 2018].

The outcomes of research in the area of experimental autoimmune encephalomyelitis (EAE), an animal model for MS, suggested more evidences. Th17 cell CD73 expression seems to be rising as the disease develops. However, CD73 loss had no impact on the emergence of EAE or the generation of Th17-associated cytokines such GM-CSF, IFN-, or IL-17. The researchers also discovered that CD73 is not necessary to either encourage or inhibit Th17 cell proliferation in vitro [Morandi F. et al., 2018]. They came to the conclusion that CD73 expression and ADO synthesis are necessary for the recruitment of lymphocytes into the CNS during EAE development because CD73 is not expressed on brain endothelial cells. Other studies demonstrated that these results were attained by stimulating CX3CL1, which is expressed at the choroid plexus. The activity of CD73 is controlled as the illness develops. This idea is further upon by the notion that these molecules serve as potential targets for cutting-edge therapeutic approaches in immune-mediated illnesses, particularly MS [Morandi F. et al, 2018].

ii. Rheumatoid Arthritis (RA)

The function of ADO and adenosinergic ectoenzymes in the context of RA has been demonstrated by a number of writers. Neutrophils and inflammatory monocytes extracted from synovial fluids of mice with collagen-induced arthritis (CIA), a well-established mouse model of RA, were shown to express CD73 and ADORA2A at significantly higher levels than controls, according to research by Flogel et al. The investigation of the CD73 mice's CIA susceptibility was the focus of a different study. Mice lacking CD73 are noticeably more vulnerable to CIA than wild-type mice. In addition, Th1 T cell responses, joint degradation, and proinflammatory cytokine production in the joints all increased. In CD73-deficient animals, a delayed anticollagen IgG response was also found, pointing

to a problem with isotype class switching. [Morandi F. et al., 2018] The authors have shown that CD73 expression on nonhematopoietic cells was crucial for preventing CIA formation.

iii. Juvenile Idiopathic Arthritis (JIA)

The persistent arthropathy known as juvenile idiopathic arthritis (JIA), which is assumed to be immune-driven, causes gradual joint degeneration if left untreated. The ability to sample cells aspirated from the site of inflammation makes this group of disorders an attractive model to study immunoregulation. This PhD study looked at the expression of CD39 and CD26 as well as the distribution and enzymatic activity of the ecto-nucleotidase CD73 to learn more about how purinergic pathway abnormalities contribute to the pathophysiology of JIA. The data reported here show that the percentage of CD73+ T and B synovial lymphocytes in JIA patients is much lower than that in patients' and healthy participants' peripheral blood lymphocytes. Although it was connected with the patient's cumulative joint count rather than the length of the disease, this reduction increased with increasing disease severity [Gordon-Smith S.B., 2015]. Adenosinergic ectoenzymes have a different function when it comes to juvenile idiopathic arthritis (JIA), a pediatric type of RA. In example, CD16CD56 NK cells isolated from synovial fluid of JIA patients showed lower expression and function of CD38 and CD73. Compared to cells derived from healthy controls, these cells showed a different kinetic for the generation of ADO and did not stop the proliferation of autologous CD4+ T cells. Other authors have shown that in JIA patients, CD19+ and CD8+ lymphocytes extracted from SF expressed less CD73 than those recovered from PB. As a result, SF CD8+ cells generated less ADO than their PB counterparts did.

There was no discovered genetic connection for NT5E (encoding CD73) that might account for the various amounts of CD73 seen in various JIA subtypes. First-line DMARD methotrexate treatment had no effect on the percentage of CD73+ peripheral blood cells, and neither did this percentage predict a patient's response to methotrexate. Low levels of adenosine in the synovium are suggested by the decline in CD73+ synovial lymphocytes and CD73 protein expression per CD73+ cell, as well as the decreased capacity to produce immunoregulatory adenosine in vitro. The perception of inadequate adenosine generation in the JIA joint and of poor inflammation attenuation is further exacerbated by CD39+ and CD73+ cells' inability to work together to convert ATP to adenosine. When cells were activated in vitro, CD73+ PBMC and isolated CD8+CD73+ T cells showed signs of downregulation. Loss of CD73+ PBMC was linked to a decreased capacity to produce adenosine. Only proliferating cells appeared to have lost CD73+ PBMC. Adenosine levels in the joint are abnormal when CD73 is downregulated, which may be a factor in the locally damaging inflammation found in JIA [Gordon-Smith S.B., 2015]. Finally, patients with an extended oligoarticular form had lower levels of CD73 expression in SF cells than patients with milder forms, suggesting that CD73 expression and ADO production are connected with disease severity because of inadequate anti-inflammatory action [Morandi F. et al., 2018].

iv. Autoimmune Uveitis (AU)

Experimental Autoimmune Uveitis (EAU), a mouse model of human Autoimmune Uveitis, has also been linked to a possible role for ADO. It has been established that suppressor antigen-presenting cells, which present autoantigens and generate ADO to activate antigen-specific T-regs, mediate the recovery from EAU. Through a PD-1/PD-L1-dependent mechanism, these inducible T-regs can, in turn, inhibit the autoimmune illness [Morandi F. et al., 2018]. Additionally, mesenchymal stem cells (MSC) have been shown to lessen the severity of the illness. This suggests that this immune-modulatory action was related to ADO, which is created by the collaboration between CD73 and CD39 and in turn abolishes T cell activities. Such therapeutic effect was reversed by pretreating MSC with a CD73 inhibitor. T cells are significant in the context of EAU because they have the ability to either increase or inhibit an adaptive immune response. According to research, CD73 was expressed by T cells at various EAU stages, and a low CD73 expression on T cells was associated with an elevated Th17 response

[Morandi F. et al., 2018]. Upon activation, CD73 expression on T cells is momentarily downregulated. Furthermore, compared to T cells separated from WT mice, T cells isolated from CD73 animals are more effective at causing a Th-17 response and eye disease in -recipient mice. On the other hand, T cell proliferation can be inhibited in vitro by T cells derived from WT mice but not from CD73 animals. These findings collectively imply that inhibiting CD73 expression on T cells may be able to regulate both their pro- and anti-inflammatory actions [Morandi F. et al., 2018].

v. Diabetes

Researchers have been employing the straightforward but unreliable classification criteria proposed by Moll and Wright for more than 30 years. Although some authors have proposed improvements to these, the most are still pending validation or call for human leukocyte antigen analysis. Recently, new criteria that take into account both clinical and radiological aspects have been created through a global initiative. Before they are fully implemented, they will need more research, but their enhanced performance ought to lead to reduced variation between study cohorts [Coates L.C. et al, 2008]. The mouse model of human T1DM known as multiple low-dose streptozotocin (MLDS)-induced diabetes has recently been linked to adenosinergic ectoenzymes and ADO. Using wild-type mice, CD39 KO mice, and mice overexpressing CD39 who were then given MLDS, the scientists specifically looked into the function of CD39. They showed that compared to WT mice, CD39KO mice developed diabetes more quickly and frequently [Morandi F. et al, 2018].

The synthesis of ADO and the activation of ADORA2A and ADORA2B, on the other hand, provided protection against diabetes in mice when CD39 was overexpressed. Previous research in this line has shown that mice lacking ADORA2A are much more likely to develop diabetes when exposed to MLDS and have hyperproliferative T cells. As opposed to wild-type mice, CD73 KO mice were shielded against MLDS-induced diabetes [Morandi F. et al, 2018]. The persistent problem of illness heterogeneity still preoccupies specialists in this area. A case can be made for keeping at least the two subgroups of peripheral and axial disease and, possibly, dividing the peripheral disease into oligo- and poly-arthritis, notwithstanding recent calls to forsake the original five subgroups [Coates L.C. et al, 2008].

2. CD73 role in inflammatory Responses

1. CD73 in immunity and inflammation

Through the conversion of ADP/ATP to AMP and AMP to adenosine, respectively, the enzymatic activities of CD39 and CD73 play crucial roles in calibrating the length, amplitude, and chemical composition of purinergic signals sent to immune cells. As a result, an ATP-driven proinflammatory environment is changed to an adenosine-induced anti-inflammatory milieu [Antonioli L., 2013]. The ability of stomach T cells to produce IFN- but little interleukin (IL)-4 is evidence that they are often phenotypically Th1-biased. Even though Helicobacter infection is strongly related with gastritis, the infection lasts a lifetime. If the infection is not treated, compensatory stimulation of regulatory T cells to protect the stomach mucosa may occur. In fact, there are now a number of publications indicating that during infection in mice and humans, Th cells resembling T-reg are present in the gastric mucosa. Additionally, Rad et al. have suggested that these T-reg are involved in persistence. Rather from completely preventing gastritis, T-reg may create enough IL-10 and transforming growth factor (TGF)- to dampen responses. In the current study, we have demonstrated that, in comparison to wild-type T-reg, T-reg generated from animals missing CD73 fail to regulate gastritis, indicating that adenosine may also contribute to the pool of mediators that give T-reg their function [Alam M.S. et al, 2009].

The pathologic context in which the CD39/CD73 pathway is integrated causes it to evolve dynamically. Inhibiting this catabolic machinery has the potential to alter the course or determine the outcome of a number of

pathophysiological events, including AIDS, autoimmune diseases, infections, atherosclerosis, ischemia-reperfusion injury, and cancer [Antonioli L., 2013]. This suggests that these ectoenzymes are novel therapeutic targets for treating a number of diseases. Four G-protein-coupled receptors mediate the many adenosine-controlled responses (A1, A2A, A2B and A3). A series of anti-inflammatory reactions are brought on by the activation of T cells' A2A adenosine receptors (A2AARs). Recent research has demonstrated that T-reg cells express CD39 and CD73, and that their presence improves T-reg cell activity by increasing adenosine synthesis. According to another research, CD4+/CD25+/Foxp3+ T-reg cells control inflammation and aid in the recurrence of Helicobacter pylori infections. During Leishmania major infection, CD4+CD25+ T-reg cell depletion improves effector T cell activation and lowers pathogen burden. Similar to this, several results indicate a link between elevation of TGF-1, Foxp3, and CD4+CD25+ T-reg cells during human malaria infection and greater Plasmodium falciparum persistence. CD73 plays a crucial part in controlling Th cell responses and H. pylori persistence. However, nothing is known about how adenosine affects how the host reacts to a Salmonella species infection. In the current study, we looked at CD39 and CD73 expression in tissues and Th cells, including T-reg cells, during mouse Salmonella infection and assessed CD73's function in controlling inflammation and bacterial load using CD73-deficient mice. [M.S. Alam et al., 2014]

After an acute kidney injury, progressive tubulointerstitial fibrosis may develop as a result of ongoing inflammation. Inflammation can be controlled by 5'-ectonucleotidase, CD73, an enzyme that changes AMP into adenosine on the extracellular surface. It is unclear how CD73 affects the progression of renal fibrosis. It has been demonstrated how renal perivascular cells lacking CD73 affect fibrosis. [Perry H.M. and others, 2019] Hospitalization and death are frequently caused by food-borne Salmonella spp. The immune system's key regulator of inflammation, adenosine, helps to prevent tissue damage when an infection occurs. ATP is converted to adenosine by CD39 (nucleoside triphosphate dephosphorylase) and CD73 (ecto-5’-nucleotidase).

After a Salmonella infection, CD73 expression in tissues and T helper cells in mice was examined. This revealed CD73's function in controlling immunological responses and bacterial clearance in CD73-deficient (CD73) and wild-type mice. The wild-type mice with the infection had decreased CD73 transcript levels. Tissues from infected CD73 animals displayed considerably higher levels of pro-inflammatory cytokines and decreased anti-inflammatory responses as compared to wild-type mice. [M.S. Alam et al., 2014]

Marked inflammatory responses are adequate to eradicate Helicobacter species infection, as seen in IL-10-deficient mice. Persistence is therefore favored by dampening these responses. In addition, compared to infection of wild-type mice, infection of CD73 animals resulted in noticeably increased inflammation and decreased H. felis colonization. These findings support the hypothesis that activation of the A2AAR by a particular agonist reduces rat gastritis. Adenosine can now be added to the group of mediators that may be able to manage gastritis brought on by a local Helicobacter species infection and, in doing so, control colonization [Alam M.S. et al, 2009]. To reduce inflammation and stop kidney fibrosis in Foxd1CreCD73 mice assessed 14 days after unilateral ischemia-reperfusion injury or folic acid treatment, perivascular cell expression of CD73 was required. Foxd1CreCD73 animals had kidneys with more collagen deposition, more proinflammatory markers (including different macrophage markers), and more immunoreactive platelet-derived growth factor receptors than CD73 mice. Administration of soluble CD73 or macrophage deletion both effectively treated kidney fibrosis and dysfunction. Compared to wild-type controls, isolated CD73 kidney pericytes exhibited an active phenotype. A wound healing response may be stimulated by CD73 in perivascular cells, which may also decrease myofibroblast transformation and affect macrophages. Perivascular cells and macrophages are coordinated by the purinergic signaling system in the kidney interstitial milieu to reduce inflammation and stop the progression of fibrosis [Perry H.M. et al., 2019].

In comparison to wild-type mice, CD73 mice showed higher inflammatory responses, were more resistant to infection, and had a much reduced bacterial load in the liver. As a result, CD73 expression reduces inflammation

during murine Salmonellosis and weakens immunity, which causes bacterial colonization to grow and the infection to last longer.

Salmonella enterobacteria are a major cause of disease and mortality in the human population. Nontyphoid Salmonella spp. are responsible for an estimated 1.0 million cases of food-borne disease each year in the United States, making them the most common cause of hospitalization and mortality [Alam M.S. et al, 2014]. Salmonella typically stays in the intestines of immunocompetent people, where it can cause a self-limiting gastroenteritis. But Salmonella can cause bacteremia and even mortality in people who have compromised immune systems, such as HIV patients and expectant mothers. A major contributor to bacterial gastroenteritis in humans, Salmonella enterica serovar Typhimurium (ST) also causes murine salmonellosis in susceptible mouse strains, which is characterized by progressive numerous microabscess forms and septicemia. Because this model closely resembles several crucial aspects of human Salmonellosis or Typhoid fever, immunity to Salmonella has been the subject of numerous investigations [Alam M.S. et al., 2014]. Salmonella infection results in a blunted early immune response that is thought to help the pathogen's long-term survival, in contrast to other Gram-negative bacteria (such as Neisseria meningitidis and Haemophilus influenza), which primarily cause acute infection and elicit strong systemic symptoms after tissue invasion. Salmonella infection would cause less systemic inflammation, which would lessen immune-mediated injury to host tissues. The beginning of a potent Th1 response is required for protective immunity in addition to the innate host responses produced by phagocyte oxidase and inducible nitric oxide synthase (iNOS). To eliminate the germs from infected tissue, a balance between T effector and regulatory T (T-reg) cell responses is essential [Alam M.S. et al., 2014]

2. CD73 inhibition and pro-inflammatory responses

Murine splenocytes were exposed to Salmonella-WCL in the absence or presence of APCP in order to analyze the role of CD73 in cytokine levels during Salmonella infection. In splenocyte populations and CD4+ cells, Salmonella-WCL treatment raised the intracellular cytokines IL17A and IFN-, and their expression was markedly enhanced by CD73-inhibition [Alam M.S. et al., 2014]. Extracellular adenosine has been linked to increasing suppressor T cells and IL-10, two anti-inflammatory compounds, in addition to lowering proinflammatory cascades. IL-10 is released from macrophages and microglia specifically in response to A2B and A2A signaling. The protective effects of extracellular adenosine on CD73 in T. gondii i.p. infection may possibly be mediated by other regulatory factors, such as interleukin-4 (IL-4) and transforming growth factor (TGF-). It would be interesting to find out whether CD73-generated adenosine encourages the production of anti-inflammatory mediators required for limiting pathology during intraperitoneal T. gondii infection [Mahamed D.A. et al, 2015].

Similar to this, elevated levels of IFN- and IL17A were discovered in the culture supernatants of splenocytes treated with Salmonella-WCL, and after CD73 was inhibited by APCP treatment, these cells greatly enhanced their production of the cytokines. As a result, Salmonella-WCL induces greater expression of pro-inflammatory cytokines in vitro when CD73 is inhibited. Since CD73 cleaves 5'-AMP to produce extracellular adenosine, the specificity of CD73 with APCP was examined by assessing the levels of pro-inflammatory cytokines in the culture supernatant of Salmonella-WCL-treated splenocytes. This functional experiment demonstrated that splenocytes that express CD73 produce adenosine, which inhibits the production of pro-inflammatory cytokines. It is understood that adenosine functions through activating adenosine receptors that are produced by immunological and inflammatory cells. Spleen tissues with Salmonella infection exhibit higher A2aAR mRNA than A2bAR. These findings support the notion that CD73-produced extracellular adenosine can operate through adenosine receptors [Alam M.S. et al., 2014]. CD73 plays a key role in the purinergic pathway that transforms proinflammatory ATP/ADP into immunosuppressive adenosine. A glycosylphosphatidylinositol-linked surface protein called CD73 is expressed on certain epithelial cells as well as certain subsets of T/B lymphocytes, myeloid cells, and vascular endothelial cells. The function of CD73 in cancer has been thoroughly explained. Numerous cancers have elevated levels of CD73, and therapies that specifically target CD73 may be able to reduce both

carcinogenesis and metastasis. Strong antitumor immunity is also present in CD73 knockout (KO) mice [Petit-Jentreau L. et al., 2015]. We assessed the cytokine expression from splenocyte populations and CD4+ Th cell populations of the infected wild-type and CD73 animals in order to further examine the function of CD73 in the regulation of inflammation in infected mice. Splenocytes obtained from infected CD73 mice had higher amounts of intracellular pro-inflammatory cytokines like IFN- and IL17A than splenocytes isolated from infected wild-type mice. IFN- and IL17A transcript responses were also noticeably higher in the spleens of infected CD73 mice [Alam M.S. et al., 2014].

Both strains of the virus caused modest to severe liver damage when both were examined histologically eight days after infection. The CD73 mice's livers showed a marginally higher level of inflammation, with clusters of lymphocytes and macrophages spreading to various foci throughout the parenchyma and occasionally being encircled by small numbers of neutrophils. Rarely, little foci of necrosis were seen in these locations. Although there was evidence of hepatocellular injury in both strains along with vesiculation, Kupffer cells enlargement, and edema, the wild-type mice's liver's inflammatory infiltration was less severe and concentrated around portal tracts. IFN, TNF, and IL-1 as well as inducible nitric oxide synthase had considerably greater mRNA expression in the liver tissues of CD73 animals (iNOS). These results support the enhanced Th responses in the spleen, showing a significant increase in the number of inflammatory foci and pro-inflammatory responses in the liver when CD73 expression was absent. Following a Salmonella infection, infected CD73 mice have heightened hepatic inflammatory responses [Alam M.S. et al., 2014]

3. Inflammation and Non-cell-bound CD73

Adenine nucleotides that are extracellular take part in cell-to-cell communication and control the immune response. Although both ectonucleotidases are infrequently co-expressed by human T cells, their cooperative action is crucial in the local synthesis of the anti-inflammatory adenosine. When T cells are activated, their CD39 expression rises and is particularly strong when inflammation is present. Contrarily, after activation, CD73 vanishes from the cellular membrane. The puzzle of both enzymes being co-expressed for the breakdown of ATP and the production of adenosine would be solved if CD73 had the potential to function in trans. The soluble form of CD73 that is enzymatically active has been described, and AMPase activity has been found in the bodily fluids of cancer and inflammatory disease patients [Tolosa E. et al., 2019]. Although the exact process by which CD73, a protein that is glycosylphosphatidylinositol (GPI)-anchored, gets released from the cell membrane is unknown, some likely candidates include metalloproteinase cleavage and shedding mediated by cell-associated phospholipases. Importantly, CD73, like many other GPI-anchored proteins, is preferentially positioned at the cell membrane in lipid rafts or detergent-resistant regions, which frequently produce extracellular vesicles (EVs). In fact, the tumor microenvironment contains CD73-containing vesicles of various sizes, origins, and immunomodulatory functions. The spectrum of action of this enzyme at sites of inflammation is expanded by the presence of CD73 as a non-cell-bound molecule. We shall talk about the physiological function of non-cell-bound CD73 in inflammation in this review [Tolosa E. et al, 2019].

By attaching to P1 receptors on immune cells, adenosine mostly produces anti-inflammatory signals. CD73 is essential for maintaining a healthy balance between immunological suppression and inflammation since it is the rate-limiting enzyme for the synthesis of adenosine. In addition to mediating lymphocyte adherence to the endothelium, CD73 has been shown to operate as a costimulatory signal for lymphocyte activation independent of its enzymatic activity. However, more recent research indicates that leukocyte adherence to endothelium is restricted by CD73-mediated adenosine synthesis and subsequent signaling through adenosine receptors. Surprisingly, CD73-deficiency prevented experimental autoimmune encephalomyelitis in mice by limiting the entry of pathogenic immune cells into the brain, but in a stroke model, it increased the size of cerebral infarcts and local leukocyte infiltration. These seemingly incongruous findings on the function of CD73 in brain inflammation may be explained by the availability of adenosine and the varied adenosine receptor activation in

chronic and acute settings, highlighting the complex roles played by CD73 and adenosine in inflammation. By enhancing endothelial barrier function, CD73 expression and adenosine signaling have also been linked to the control of vascular permeability [Tolosa E. et al., 2019].

While the expression of CD73 on endothelium has been observed in many different species, immune cells express CD73 differently depending on the species. In humans, CD73 is expressed on the majority of B cells (definitely on all mature naive B cells) and on various T cell subsets, including innate-like T cells, naive cells in the CD8 compartment, and a tiny percentage of memory CD4 T cells. On most T cells, including T-regs, NK cells, and peritoneal macrophages in mice, CD73 is present. In the B cell compartment, it is selectively expressed in mature class-switched and germinal center B cells. Interestingly, despite murine T-regs constitutively expressing both ectoenzymes, human conventional T cells in the periphery seldom co-express CD39 and CD73, and human T-regs rarely express CD73. While CD73 expression is modest, CD39 is increased on T cells in the inflamed joints of arthritis patients. As a result, human conventional T lymphocytes that have been activated in vitro exhibit an overexpression of CD39 and a loss of CD73 from the cell membrane. Interestingly, a subgroup of Th17 cells with suppressive properties co-express CD39 and CD73. Underscoring their significance for the regulation of inflammation in the gut, these cells were discovered to be diminished in individuals with inflammatory bowel disease. They are predominant in the lamina propria.

Due to the rarity of their co-expression in T cells, it is possible that the AMPase activity of CD73 is supplied in trans by nearby cells of different lineages. The enzymatic activities of CD39 and CD73 are complementary for the production of adenosine and subsequent control of the inflammatory response. We know from the tumor microenvironment that CD73-positive extracellular vesicles (EVs) contribute to the suppression of anti-tumor immune responses and that EVs derived from murine regulatory T cells exhibit AMPase activity. However, how CD39-expressing T cells are endowed with AMPase activity in the context of inflammation is not fully understood. The loss of cell surface CD73 in T cells from synovial fluid from arthritic patients or after in vitro stimulation suggests that CD73 may be shed off the cell surface, either as a soluble molecule or in the form of vesicles. Vesicular release is accelerated with cell activation. By creating an adenosine-rich anti-inflammatory milieu, non-cell-bound and enzymatically active CD73 distributes at the sites of inflammation and modifies the immune response. We shall talk about the physiological function of non-cell-bound CD73 in inflammation in this review [Tolosa E. et al, 2019].

A GPI-anchor holds CD73, a 71 kDa homodimer, to the plasma membrane. There are three different forms of the protein that come from the GPI-anchored form: a soluble version, a membrane-bound phospholipase C-sensitive form, and a membrane-bound phospholipase C-resistant form. Human placental extracts' supernatant included soluble and enzyme-active CD73 that had the same affinity for AMP as the membrane-bound version. Additionally, AMPase activity in human plasma, serum, and vitreous fluid can be selectively inhibited by the ecto-5’-nucleotidase inhibitor adenosine 5’-(-methylene)-diphosphate (APCP), demonstrating the existence of soluble CD73 [Tolosa E. et al., 2019].

Not all ectoenzymes found in soluble form in peripheral blood are CD73. Human plasma also contains ATP-regenerating kinases, ATP-degrading enzymes like ENPPs and CD39, alkaline phosphatase, adenosine deaminase (ADA), CD38, and other purine-metabolizing enzymes. This suggests that there is a complex network of enzymatically active molecules that shifts the balance of purinergic signaling and, in turn, modulates the immune response [Tolosa E. e Both proteolytic cleavage and endogenous phospholipase hydrolysis of the GPI-anchor can cause CD73 to shed. Myo-inositol, a component of the GPI-anchor attached to the protein following phospholipase shedding, was present in soluble CD73 from human placenta, indicating that endogenous phospholipase C or D was responsible for the protein's release from the membrane. Only phospholipase C's cleavage of the GPI-anchor preserves the cross-reacting determinant (CRD) epitope between these two phospholipases' distinct sites of cleavage. This epitope was found in isolated bovine sCD73 using an antibody that detects the CRD, indicating to phospholipase C-mediated CD73 shedding. The partial resistance of

lymphocytic and placental CD73 to phospholipase C cleavage suggested the presence of a CD73 variant that is not GPI-linked. However, it was discovered that none of the cloned CD73 cDNAs from various species encode for a variation with a typical membrane domain. It's interesting to note that there is proof that the inositol-palmitoylation group's is the cause of the observed resistance to phospholipase C-mediated shedding. In order to regulate the shedding of CD73, palmitoylation of the GPI-anchor may serve as a regulatory mechanism. It should be noted that palmitoylated GPI-anchored proteins are still susceptible to phospholipase D, and that mammalian plasma contains extracellular phospholipase D that can shed CD73. The fact that alkaline phosphatase, another enzyme that may produce adenosine from AMP, is likewise GPI-anchored suggests that phospholipase-mediated cleavage may be a common way for immune cells to avoid autocrine adenosine-mediated suppression [Tolosa E. et al, 2019].

A soluble version of CD73 can also be produced through proteolytic cleavage of the enzyme in addition to phospholipase-mediated shedding. It has been demonstrated that the matrix metalloproteinase 9 (MMP-9) cleaves CD73 off the membrane of stimulated mouse retinal pigment epithelium cells. However, the sCD73 that was produced lacked any enzymatic activity. In contrast, bull seminal plasma contained an active version of sCD73 produced by proteolytic cleavage and devoid of the GPI-anchor. Since it has a lower affinity for AMP than the membrane-bound variant, this soluble protein differs from the GPI-anchored form in its posttranslational modifications, aggregation patterns, and enzymatic activity [Tolosa E. et al, 2019]. Lower catalytic effectiveness of the membrane-bound versions of CD73 was discovered when AMPase activity was directly compared between lymphocyte membrane-bound CD73, GPI-anchored CD73 placed into an artificial lipid bilayer, and sCD73. Additionally, ectonucleotidase activity is increased as a result of CD73 being released from the membrane by phospholipase C. As a result, CD73's enzymatic activity is increased as a result of its release from the cell membrane via phospholipase [Tolosa E. et al., 2019].

The fact that GPI-anchored proteins are enriched in particular cell surface domains, such as the so-called lipid rafts or detergent-resistant membranes, which act as platforms for signal transduction, rather than being evenly distributed throughout the cell surface, is a defining characteristic of these proteins. Probably because they are inhabitants of these particular domains, GPI-anchored proteins are also found in EVs. EVs are lipid bilayer vesicles that are secreted by the majority of cell types and are capable of carrying a variety of cargoes, including proteins, lipids, mRNA, non-coding RNA, and DNA. Exosomes (which have an endosomal origin) and ectosomes, or microvesicles/microparticles, which are produced by vesicle shedding at the plasma membrane and include apoptotic bodies (which are produced from the plasma membrane of cells undergoing apoptosis), or large oncosomes, can be distinguished according to their origin (originated from the plasma membrane of cancer cells). EVs range in size from 30 nm to different m, depending on where they come from. In EVs, in particular exosomes, produced from cancer cells, regulatory T cells, mesenchymal stem cells, as well as from human plasma, CD73 protein and AMPase activity have been found [Tolosa E. et al, 2019]. Furthermore, since the existence of EVs was not taken into account in those investigations, it is unclear if the soluble CD73 (or its enzymatic activity) in human bodily fluids described in other studies is actually soluble or vesicle-associated, or both.

Different cancer cell lines' extracellular vesicles co-express CD39 and CD73 and have the ability to hydrolyze ATP into adenosine, altering the tumor microenvironment and T cell activity without coming into touch with immune cells directly. Additionally, colon cancer patients' blood levels of B cell-derived CD39+CD73+ EVs are higher, and these EVs convert tumor-derived ATP to adenosine, limiting CD8 T cell anti-tumor responses. The effect of CD73 inhibition on T cell function was significant in both experiments. In co-cultures with CD39+ T-regs, vesicles isolated from plasma of healthy donors or patients with neck squamous cell carcinoma displayed AMPase activity and converted ATP to adenosine, proving that co-expression of CD39 and CD73 on the same cell is not required to give T-regs an adenosine-mediated suppressive function. As a result, CD73's enzymatic activity in EVs considerably impedes the immune system's ability to fight cancer [Tolosa E. et al., 2019].

EVs produced from activated murine T-regs are CD73-positive, convert AMP to adenosine, and mediate immunological suppression. Murine T-regs express both CD39 and CD73. Human T cells that have been activated, whether regulatory or conventional, express CD39 but not CD73. While ATPase and ADPase activities are primarily mediated by cell-associated enzymes, the analysis of the enzymatic activity responsible for ATP degradation in human blood revealed that the enzymes present in body fluids, such as plasma, are what carry out the final step and convert AMP to adenosine. In fact, arthritic patients' synovial fluid supernatants can be used to evaluate CD73-specific AMPase activity. Synovial fluid T cells from arthritis patients have high levels of CD39 expression and robust AMP production in comparison to the cell-free moiety, but low levels of CD73 and subsequently subpar adenosine synthesis. The missing AMPase activity might theoretically be supplied by soluble CD73 or CD73-containing EVs that are locally released upon activation, completing the ATP degradation cascade to adenosine. Furthermore, it is conceivable that the AMP produced by the producing cell diffuses briefly to a CD73-expressing cell in the extracellular space where it is digested. As a result, co-expression of the ATP- and AMP-degrading enzymes in the same cell is not always necessary for the production of adenosine. Additionally, since AMPase activity can be provided in trans, differences in CD73 expression on immune cells between humans and mice may not be of great significance. It should be noted that less research has been done on the roles that non-cell-bound CD38 and ENPPs play in the production of AMP, and that only recently have the enzymatic activity of these enzymes been reported in EVs derived from multiple myeloma cells [Tolosa E. et al, 2019].

At locations of inflammation, active or dying cells release ATP and NAD+. Parallel to this, purinergic enzymes CD39 and CD38 are expressed more strongly by activated immune cells, and CD73 is lost from the cell membrane, either as a soluble molecule or as a component of EVs. Local stromal cells with CD73-containing vesicles can also be released, including endothelial and mesenchymal stem cells. Adenosine is produced when ATP and NAD+ are broken down either intracellularly by enzymes that are bound to cells or extracellularly by soluble or vesicular enzymes, and this substance inhibits the immune response by binding to P1 receptors on immune cells. The non-cell-bound form of CD73 has two advantages over cell-associated CD73: first, it extends the range of enzymatic activity beyond the cell membrane; and second, it shields the activated "donor" cell, which loses membrane CD73, from pericellular adenosine, which could prematurely shut down its effector function. Even while membrane-bound CD73 is more active enzymatically than its soluble counterpart, the release of membrane-bound enzymes in the form of vesicles has a number of benefits: First, the half-life is prolonged because degradation is avoided; second, the enzymatic activity is more effective due to the concentration of the enzyme in microdomains; third, distant transport is facilitated, allowing for systemic modulation; and, fourth, the fusion of the EVs with the plasma membrane of the target cells can give them the missing enzymatic activities [Tolosa E. et al, 2019].

There are various checkpoints that regulate the adenosinergic pathway: First, the rate of extracellular adenosine synthesis at the site of inflammation is controlled by the regulation of CD73 expression and shedding. Adenosine availability is further influenced by its degradation to inosine, which is mediated by adenosine deaminase, and by cellular uptake aided by nucleoside transporters. It is important to note that higher concentrations of non-cell-bound CD73 and of its substrate AMP do not necessarily result in high extracellular adenosine. Furthermore, it has been shown in the supernatants of inflamed ileum organ culture in a model of postinflammatory ileitis that high quantities of ATP and ADP can decrease CD73 enzymatic activity. Finally, the amount of accessible adenosine determines whether the target cells' high-affinity (A2A) or low-affinity (A2B) receptors are active. Adenosine signaling is further modulated in hypoxic settings, when A2B receptors are particularly upregulated and quiet A2A receptor signaling.

When is the presence of AMPase desirable? Since tonic adenosine signaling is essential for the preservation of the naive T cell pool, it is present in resting T cells. Immune control is essential for the contraction of the immune response after an infection or sterile inflammation as well as for preventing immunopathology. Many immune cell types, aside from FoxP3+ T-reg cells, can develop regulatory properties. However, too much regulation can result in insufficient immune responses, which can then promote pathogen proliferation or hamper tumor

management. Therefore, a method that works on several cell types and can be readily modified constitutes a significant advantage. Since inhibitory P1 receptors are extensively expressed on immune cells and the enzymatic activities for adenosine synthesis and degradation are swiftly controlled, adenosine-mediated immune suppression satisfies these requirements. Adenosine is further supported as the optimal immune response regulator by the discovery that the enzymes responsible for its production are easily shed from the cell membrane and can be transported to function on other cell types. In fact, it was discovered that T cell-mediated adenosine synthesis was increased and helpful for recovery after myocardial infarction. Similar fluctuations in systemic adenosine have been observed in people following strokes, and significant AMPase activity has been found in neonatal plasma, perhaps assuring tolerance to the novel microbial environment. Having a better understanding of how systemic AMPase activity is controlled can help guide therapeutic action. The systemic use of CD73 to reduce inflammation is hindered by vasodilation and a resulting drop in blood pressure in response to adenosine receptor interaction in the vasculature, even if blocking CD73 appears to be a promising method in cancer immunotherapy. As elegantly demonstrated by Flögel et al. in a model of arthritis, methods that limit adenosine signaling to the site of inflammation must thus be put in place. Locally administered EVs with the CD73 gene may offer an additional therapeutic approach. [Tolosa E. et al, 2019]

4. Other Inflammatory Diseases 311

Autoimmune diseases and a wide range of other disorders and ailments that are marked by inflammation are examples of inflammatory diseases. This includes preperfusion injury, inflammatory disorders connected to transplant, allergies, asthma, celiac disease, glomerulonephritis and other renal diseases, hepatitis, and inflammatory bowel disease. Consideration should be given to the function of ADO in the context of skin allergies, renal inflammatory disorders, and graft-versus-host disease (GvHD) [Morandi F. et al, 2018].

i. CD73 in Inflamed Human Skin

Leukocyte adherence to endothelial cells is mediated by CD73, which is strongly expressed on endothelial cells. As a result, CD73 controls how these later cells enter the vascular network and are subsequently recruited to the inflammatory site. Additionally, the endothelial barrier function is supported by the anti-inflammatory effects of ADO generated by CD73+ endothelial cells. In the context of idiopathic and allergic skin illnesses, Arvilommi et al. looked into the involvement of CD73 in the migration of activated lymphocytes to inflamed skin. They showed that CD73 is also expressed on activated lymphocytes, and that the majority of skin-homing lymphocytes also expressed cutaneous lymphocyte antigen in addition to CD73 (CLA). Additionally, peripheral blood lymphocytes treated with an anti-CD73 monoclonal antibody experienced a significant decrease in their ability to bind to the vascular endothelium of the inflamed skin, suggesting that CD73 plays a role in the attraction of activated lymphocytes in this situation [Morandi F. et al, 2018].

ii. CD73 in Renal Diseases

Researchers have looked into the crucial function of CD73 on endothelial cells in the regulation of leukocyte trafficking in mice with renal damage. In actuality, CD73 mice displayed autoimmune inflammation with glomerulitis and peritubular capillaritis, immunoglobulin and complement deposition in the glomeruli, as well as increased lymphocyte and macrophage infiltration in the interstitium. Additionally, increased blood levels of proinflammatory cytokines and chemokines were linked to vascular inflammation. Last but not least, CD73 mice showed fewer podocytes and endothelium fenestrations. According to these findings, autoimmune inflammation and impaired renal function are related [Morandi F. et al., 2018].

iii. Graft-versus-Host Disease

Through the secretion of soluble immunosuppressive chemicals such TGF-1, HGF, IDO, PGE2, IL-10, and HLA-G, MSC are able to regulate immune activation. MSC have been suggested as a therapeutic intervention in the management of severe acute GvHD due to their immunosuppressive capability. Sangiorgi et al. recently reported that bone marrow-derived MSC I raised CD73 and CD39 expression on their surface and (ii) showed increased immunosuppressive activities upon activation with a toll-like receptor 9 agonist [Morandi F. et al, 2018]. As a result, they draw the conclusion that ADO produced through the CD39/CD73 pathway may also contribute to the immunomodulatory activity of MSC. Human gingival mesenchymal stem cells (GMSCs) have similar abilities to MSC in that they can inhibit immunological responses and T cell-mediated CIA in rats. In vitro experiments have shown that GMSCs can inhibit PBMC and T cell growth. In a mouse model of GvDH, GMSCs can prevent the engraftment of human cells and increase the lifespan of mice when cotransferred with PBMC. It has been established that the enzymatic activity of CD39 and CD73 as well as the synthesis of ADO contribute to the GMSC-mediated immunosuppression. These cells provide a possible clinical treatment option for autoimmune and GvHD conditions [Morandi F. et al., 2018].

The observation that T-regs from CD73 KO mice are less able to reduce GvHD mortality than WT T-regs supports the function of CD73 and ADO in the management of GvHD. Further evidence that ADO is implicated in this process through its interaction with the A2a receptor comes from the fact that blockage of the ADO A2a receptor made GvHD worse. Last but not least, CD73 inhibition caused alloreactive T cells to grow, which aggravated GvHD and improved the graft-versus-tumor impact. According to these findings taken together [Morandi F. et al., 2018], inhibiting CD73 activity may be important in the context of BM transplantation.

3. CD73 role in infectious diseases

Recently, it has been recognized that CD73 may have a role in the host's defenses against microbial infection. It is now understood, for instance, that a symptomology connected to some intestinal infections may be connected to extracellular nucleotide metabolism. In fact, Crane et al. demonstrated that damage caused by enterpathogenic E. coli infection of the epithelium liberates extracellular ATP and produces adenosine via CD73-dependent pathways. Adenosine is a strong secretagogue, as was mentioned earlier, and in this situation it may encourage intestinal symptoms of secretory diarrhea. Similarly, although most studies have been indirect, some evidence suggests that CD73 may help with microbial responses mediated by Toll-like receptors (TLRs). Although numerous cell types express the necessary components for TLR-mediated signaling, monocytes/macrophages and CD73 dendritic cells have been the subjects of the most extensive research in the field of TLR signaling pathways. This is because different lineages of dendritic cells seem to express CD73, and it has been proposed that this expression may control a variety of processes, from angiogenesis to B-cell maturation. Additionally, CD73 might influence how the body reacts after a viral infection. For instance, Kas-Deelen et al. recently revealed that cytomegalovirus (CMV) infection increases the expression and activity of both ecto-ATPase(s) and CD73 in endothelial cells. [SP Colgan, 2006]

i. Salmonella infection

Although CD73 transcript levels are increased in hypoxia and inflammation, nothing is known regarding the expression of mRNA or proteins in response to infection. The levels of CD73 mRNA transcript in the liver and spleen of wild-type (C57BL/6) mice fell dramatically after an oral Salmonella infection. Intestinal data showed a comparable decline. Recent data suggests that CD39 and CD73 have a role in the regulation of CD4+ T cell function. Both CD4+ Th cells and uninfected or dormant splenocytes highly express CD73. When splenocytes were exposed to Salmonella WCL, expression of CD39 and CD73 in lymphocytes and CD4+ T cell populations was reduced. Together, these findings indicate that CD39 and CD73 are expressed by murine tissues and Th cells to

produce adenosine locally, and that Salmonella infection significantly reduces both CD39 and CD73 expression [Alam M.S. et al., 2014].

In wild-type and CD73 mice, salmonella infection marginally increased the percentage of T-reg cells (CD4+CD25+), whereas the percentage of T-reg expressing CD73 dramatically reduced after infection. T-reg cells from CD73 mice may not operate adequately after infection if CD73+ cells are not present. According to a prior study, CD73 mouse-derived T-reg cells are ineffective at controlling T effector cells. When splenic tissue and splenocytes were examined for anti-inflammatory cytokine expression, it was shown that infected CD73 animals did not significantly express more IL-10 than infected wild-type mice. Additionally, the IL-4, TGF-1, and IL-13 mRNA response, which is similarly implicated in controlling T-reg cell-mediated anti-inflammatory responses, was markedly diminished in the infected CD73 animals. [M.S. Alam et al., 2014]

The function of CD73 in the host response to infection was investigated in CD73 mice because suppression of CD73 by APCP improved cytokine responses after stimulation with Salmonella-WCL. Salmonella was administered at varying doses to CD73 and wild-type mice, and body weight was tracked throughout. Indeed, throughout the Salmonella infection trials, we saw that CD73-deficient animals had considerably fewer severe clinical symptoms than ST-infected wild-type mice [Alam M.S. et al, 2014].

We expected that the adenosine-mediated modulation of Th cells would contribute to Salmonella persistence because CD73 gene deletion in mice gives resistance against ST infection and results in dramatically increased pro-inflammatory responses in these tissues. In comparison to wild-type mice, the CD73 mice had much reduced levels of Salmonella colonization in the liver. [M.S. Alam et al., 2014]. An proper host response involving the coordinated activity of innate and adaptive immunity is necessary for the control and clearance of intracellular infections. In the mouse model of typhoid, the removal of Salmonella necessitates the generation of IFN- and IL-17 by Th17 cells, both of which are crucial for enhancing the inflammatory responses in the liver and stomach. Compared to wild-type mice, CD73-deficient mice also exhibited higher survival rates, lost noticeably less body weight, and were more adept at eradicating Salmonella infections in the liver and spleen. [M.S. Alam et al., 2014]. The capacity of T-reg cells to produce adenosine from ATP and ADP can considerably aid in the regulation of effector cells during infection-induced inflammation since CD73 is a rate-limiting factor for extracellular adenosine synthesis. The idea that adenosine contributes to the pool of mediators necessary for efficient T-reg cell function is further supported by earlier research showing that T-reg cells generated from mice lacking CD73 are less effective at controlling inflammation than wild-type T-reg cells. In the current work, we found that infected CD73 mouse tissues produced less of the anti-inflammatory cytokines IL-10, IL-4, and TGF-1. This finding further shows that the T-reg cells from these animals do not release enough IL-10 and TGF-1 to suppress the pro-inflammatory response. [M.S. Alam et al., 2014]

Adenosine A2AAR and A2BAR are critical regulators of morbidity and mortality during polymicrobial sepsis and infection, in addition to our results that CD73 modulates immunity and exacerbates Salmonellosis. These studies lend credence to the idea that adenosine plays a substantial role in controlling illness in infected mice.

Hasko et alreport.'s of an opposing effect of CD73 during sepsis serves as an illustration of the complexity of adenosine function. Recent research by Mohamed et al. demonstrated that CD73 mice are resistant to Toxoplasma gondii-induced death because the host has poor parasite differentiation. The current discovery that CD73 inhibits inflammation and hinders bacterial clearance is in line with a prior study that found that CD73 inhibition or A2AAR-deletion enhanced pro-inflammatory responses in human Th cells as well as increased gastritis in an animal model of H. pylori infection. Additionally, CD73 may have a role in non-catalytic co-stimulation and/or adhesion-related processes. The current study's findings and the increased inflammation in CD73-infected mice support the idea that the lack of CD73 increases T cell activation via impairing the production of adenosine rather than by disrupting activation signals [Alam M.S. et al., 2014].

Salmonella infection decreased CD73 expression in a tissue, indicating the host was trying to encourage adaptive immune responses to get rid of the infection. This reaction may be necessary to prevent the suppressive effects brought on by the production of adenosine during T cell activation. The mechanism underlying the possible decrease in CD73 expression on T-reg in response to infection is still unknown. One theory is that the cytokine environment created by Salmonella infection influences CD73 expression. For instance, a decline in TGF-1 would eliminate a strong stimulus for CD73 production. If this effect is intended to limit bacterial load, it is less drastic than the absolute impairment of this system in CD73-deficient animals, which reduces bacterial burden and improves survival. This is because effector Th cells and other cells that express CD73 might also add to the adenosine pool, which works to reduce host response. The anti-microbial responses would be inhibited by this pool, which would thus encourage infection [Alam M.S. et al, 2014].

ii. Mycobacterium tuberculosis

The immune system requires protections that allow for an efficient reaction against a disease while preventing collateral tissue damage caused by the immune system. One such mechanism is the purinergic pathway, which controls inflammation with precise precision by detecting environmental nucleotides. Adenosine is immunosuppressive, while extracellular ATP is thought to be a danger signal that triggers a proinflammatory response. This route places CD73, also known as ecto-5'-nucleotidase, in a key position because it is the primary enzyme in charge of producing adenosine from ATP. In tuberculosis, an illness marked by an immune response that is damaging to the host and incapable of wiping out Mycobacterium tuberculosis, CD73 plays a role. In vitro and in vivo, CD73 controls the immune response to M. tuberculosis infection. When stimulated with ATP, mycobacterium-infected murine macrophages derived from CD73 KO mice release more of the cytokines keratinocyte chemoattractant (KC), tumor necrosis factor alpha (TNF-), and interleukin-6 (IL-6) and less of the cytokine vascular endothelial growth factor (VEGF) than those derived from wild-type (WT) mice. Without altering bacterial development or spread in vivo, CD73 restricts the initial flow of neutrophils to the lungs. According to Petit-Jentreau L. et al. (2015), CD73 optimizes immune responses against mycobacteria.

One of the most widespread infectious illnesses in the world, tuberculosis (TB) claimed 1.5 million lives and caused 9 million new cases in 2014. The ability of Mycobacterium tuberculosis, the disease's etiological agent, to survive and reproduce inside phagocytes, is crucial to its success. This intracellular pathogen has created a variety of strategies to evade macrophages' bactericidal actions and control host immunological reactions. M. tuberculosis specifically suppresses autophagy, phagosome acidification, inflammasome activation, and inhibits apoptosis. An key aspect of TB is cell death. The host response coordinates the creation of granulomas, which are structured cellular masses that are phagocytosed by alveolar macrophages. By causing the infection of freshly recruited nonactivated phagocytes, these structures paradoxically appear to encourage the infection while simultaneously containing it. The center of the granuloma becomes necrotic and disintegrates, releasing the germs into the extracellular environment if the host is unable to regulate M. tuberculosis. However, necrosis also causes the release of the intracellular material into the milieu, allowing M. tuberculosis to escape from an innate immune defense mechanism and cause the infection of bystander phagocytes [Petit-Jentreau L. et al, 2015].

The immune system is affected in a number of ways by the presence of intracytoplasmic molecules in the extracellular space. Numerous intracellular molecules have been identified as danger indicators or damage-related chemical patterns (DAMPs). It is generally known, for instance, that the purinergic pathway affects immunity. Numerous inflammatory reactions are triggered by ATP released from dying cells or through pannexin and connexin hemichannels, including neutrophil granule release, T-cell activation, macrophage cytokine and chemokine secretion, the production of reactive oxygen or nitrogen species, and dendritic cell maturation and migration. In the extracellular environment, ecto-ATPases of the ectonucleoside triphosphate

diphosphohydrolase family quickly convert ATP to ADP and AMP. Ecto-5′-nucleotidase then converts AMP to adenosine (CD73). Adenosine is thought to be anti-inflammatory in contrast to extracellular ATP. For instance, the extracellular adenosine A2A receptor's signaling prevents neutrophils and macrophages from producing free radicals or secreting inflammatory cytokines, and it suppresses the activation of Th1 and Th17 cells. Adenosine A2A receptor activation dampens the expression of inflammatory genes and boosts the expression of genes involved in tissue healing in human macrophages infected with M. tuberculosis. et al. [Petit-Jentreau L. 2015]

Immune responses need to be carefully controlled to prevent too much tissue damage. One such regulation route is purinergic signaling. Adenosine is thought to reduce inflammation, but extracellular ATP is thought to promote it. In the current investigation, we assessed CD73's function as the rate-limiting enzyme that turns AMP into adenosine. In vitro and in vivo, in M. tuberculosis-infected mice, CD73 alters how M. tuberculosis-infected BMMs react to ATP stimulation. BMMs from CD73 KO mice that were infected with M. tuberculosis released more KC, TNF-, and IL-6 than BMMs from WT mice. After 21 days post-infection, CD73 KO mice had higher levels of these cytokines in vivo than WT mice (with the exception of VEGF). However, neither in vivo nor in vitro mycobacterial development is impacted by CD73 loss. In contrast, CD73 reduces bacterial clearance in the spleens and livers of mice infected with H. felis and S. enterica serovar Typhimurium, respectively. In reality, CD73 appears to play only a temporary function in the early homing of neutrophils and other innate immune cells to the lung parenchyma and other tissues injured by M. tuberculosis infection. Our findings demonstrate that CD73 inhibits KC production in BMMs and in vivo, which likely explains why CD73 KO mice with M. tuberculosis infections had higher neutrophil recruitment rates [Petit-Jentreau L. et al., 2015].

Neutrophils' function in tuberculosis is still debatable and appears to have two opposing sides. Although the bacillus easily infects neutrophils, it hasn't been proven that this directly kills M. tuberculosis. However, dendritic cells' ingestion of apoptotic neutrophils makes it easier for CD4+ T lymphocytes to get activated and macrophages' phagocytosis of apoptotic neutrophils reduces the survival of intracellular microorganisms. Neutrophils play a major role in immune-mediated lung injury during M. tuberculosis infection in vivo. In vulnerable mouse strains, neutrophils are recruited in large numbers; their reduction has a protective effect and reduces the bacterial load [Petit-Jentreau L. et al, 2015].

The purinergic pathway, specifically CD39 and CD73, has an impact on the recruitment of neutrophils to the site of inflammation. In mice lacking CD39 or CD73, as well as in mice given particular inhibitors of these two enzymes, neutrophil trafficking after lipopolysaccharide (LPS)- or bleomycin-induced lung damage is very active. Additionally, neutrophil influx has been seen in T. gondii-infected CD73 KO mice. Although CD73 is extensively expressed by immune cells, including macrophages, its surface expression on endothelial cells may play a crucial role in the regulation of neutrophil inflow and tissue damage during M. tuberculosis infection. Adenosine is created by CD39 and CD73 on the surface of endothelial cells during hypoxia, which promotes the endothelium barrier's functions and starts an anti-inflammatory reaction. In fact, the microvascular endothelium responds to the activation of neutrophils via the adenosine A2A and A2B receptors as an antiadhesive signal.

There are concerns concerning CD73's potential function in humans. Contrary to human tuberculosis granulomas, mouse granulomas are typically not necrotic or hypoxic. Extracellular nucleotides are abundant during necrosis, and hypoxia controls CD73 expression. Thus, additional research is required to clarify the function of CD73 during tuberculosis, such as nonhuman primate investigations [Petit-Jentreau L. et al, 2015].

iii. Toxoplasma gondii Infection

An obligate intracellular protozoan pathogen with a wide host range and tissue tropism, Toxoplasma gondii. T. gondii tachyzoites penetrate host cells after infection and multiply quickly before activating a powerful immune response that reduces the parasite population by directly destroying it. Interleukin-12 (IL-12) priming and subsequent interferon gamma (IFN-) release by both innate and adaptive immune cells constitute the majority of the immunological response. This balance may highlight variations in genetic vulnerability to toxoplasmosis among various hosts, even while a strong immune response is necessary for limiting parasite-induced disease [Mahamed D.A. et al, 2015].

However, nothing is known about CD73's function in infectious illnesses. In comparison to wild-type (WT) mice, CD73-deficient animals are more resistant to Helicobacter felis and Salmonella enterica infections and exhibit a more pronounced inflammatory response. These findings are in contrast to those made after toxoplasma gondii infection. CD73 KO mice are resistant to chronic toxoplasmosis in the central nervous system following oral infection with T. gondii, but they are extremely vulnerable to immune-mediated harm following intraperitoneal injection of the parasites. In a sepsis scenario, CD73 KO mice likewise had a significant mortality rate. There has never been research done on CD73's function in TB. Here, we investigated the impact of this crucial purinergic pathway component on inflammatory responses during the progression of M. tuberculosis infection in mice using both in vitro and in vivo models. Our findings might make it easier to create fresh TB prevention plans [Petit-Jentreau L. et al., 2015]. Inflammation is brought on both locally and systemically by infections with organisms like T. gondii. Even though inflammation is required to remove an infection, the host frequently pays a price for it. Inflammation can cause serious collateral tissue damage if it is not controlled. The local immune response is controlled by the autocrine and paracrine production of ATP and its metabolites through the well-conserved purinergic signaling system. ATP from damaged cells, platelets, and cells under oxidative stress is released into the extracellular space during an inflammatory reaction. Through the attraction and activation of immune cells such T cells, neutrophils, and macrophages, this extracellular ATP enhances the inflammatory immune response [Mahamed D.A. et al, 2015]. The ectoapyrase CD39 dephosphorylates ATP to produce ADP and AMP. Ecto-5'-nucleotidase CD73 further converts AMP to extracellular adenosine. Adenosine from extracellular sources controls the immune response to limit excessive cellular damage. Local tissue damage can be remedied by local cells that express any of the four G-protein-coupled adenosine receptors (A1, A2A, A2B, and A3). The responding cell and the adenosine receptor(s) it expresses are likely to influence the sort of response that occurs after adenosine receptor (AR) activation. In contrast, adenosine signaling through the A2A receptor inhibits adhesion whereas the A1 receptor stimulates neutrophil chemotaxis to infection sites and their attachment to inflamed endothelium. Adenosine is crucial for the function of T regulatory cell suppressors and the production of anti-inflammatory cytokines by preventing the monocytes and dendritic cells from producing proinflammatory substances such tumor necrosis factor alpha (TNF-), IL-1, and reactive oxygen species (ROS). Adenosine controls inflammation as a result, and it also serves as a cell damage signal to encourage cell migration to locations of tissue injury. et al., 2015; Mahmoud D.A.

It is widely known that extracellular adenosine is involved in the control of local inflammation. Indeed, after a variety of inflammatory stressors, mice lacking the CD73 gene show multiple abnormalities in immunoregulation. Sepsis, inflammatory bowel illness brought on by dextran sulfate sodium (DSS), lung injury, and tissue ischemia, hypoxia, and inflammation are all more common in CD73 animals. However, CD73 inhibition or deletion may have therapeutic value in the treatment of cancer, MS, and persistent Toxoplasma gondii infection. Determining the function of this pleiotropic enzyme in immune response and inflammation may therefore provide intriguing therapeutic targets [Mahamed D.A. et al, 2015]. Surprisingly, adenosine and other purines play a significant role in T. gondii biology. T. gondii, like other apicomplexa, cannot synthesis its own purines, hence it must rely on host purines like adenosine to successfully complete its life cycle. The central nervous system (CNS) tissue had a deficiency in parasite differentiation, which was the cause of this resistance. The crucial function of CD73-

generated adenosine in controlling the immune response to T. gondii infection in an acute systemic model of toxoplasmosis is identified for the first time in this study [Mahamed D.A. et al., 2015].

Mice suffer significant tissue damage as a result of intraperitoneal T. gondii infection due to the quick immune cell infiltration and pronounced immunological response. The genetic makeup of the host and the parasite affects how an infection develops. A proinflammatory, Th1-skewed immune response to T. gondii predominates, and while this response is essential for parasite clearance and resistance, it can also be harmful to the host if not strictly controlled. The ectoenzyme CD73 was essential for preventing immunopathology and ensuring the survival of mice that had been intraperitoneally infected with T. gondii. The production of extracellular adenosine, which stopped a proinflammatory cascade, was the mechanism by which CD73's protective actions were carried out. Proinflammatory mediators such IL-1, TNF-, and nitric oxide, all of which are known to cause bystander immunopathology, were also released at higher amounts by cells from CD73 mice. In light of this, the dysregulation of cytokines that we noticed in the absence of CD73 may encourage collateral tissue damage during the early stages of an infection. et al., 2015; Mahmoud D.A.

In CD73-deficient animals, the main receptor involved in protection against hyperinflammatory cellular infiltration was the A2A adenosine receptor. A lack of extracellular adenosine may result in a proinflammatory phenotype in animals lacking CD73, either directly or indirectly through the accumulation of ATP, which is known to act as a danger signal and induce inflammation. A decrease in CD73 enzymatic activity may result in both an upstream ATP buildup and adenosine depletion. It is known that ATP activates the NLRP3 inflammasome through P2X receptor signaling, which causes pro-IL-1 to be processed by caspase-1 and released as active IL-1. It's plausible that in CD73-deficient mice, an accumulation of ATP could cause inflammasome activation through P2X7 signaling or a lack of adenosine's antagonistic effects, which could account for the observed rise in IL-1 levels. Adenosine receptor signaling, at least in the early stages of infection, appears to play a major role, according to the findings with A2A receptor knockout mice, which appear to largely reproduce the susceptibility seen in CD73 mice. It is likely that the delay in weight loss seen in A2A receptor knockout mice indicates a role for the A2B receptor early during infection [Mahamed D.A. et al, 2015]. This is because these mice have a functional A2B receptor. The limiting effect of adenosine in systemic inflammation brought on by T. gondii i.p. infection is mostly due to the A2A adenosine receptor. Similar to mice lacking CD73, A2A receptor-deficient mice also demonstrated immunopathology. For the immunological response to T. gondii to be suppressed during its acute phase, adenosine receptor signaling is essential. For instance, NECA, a nonhydrolyzable adenosine analogue that does not take part in the purine salvage pathway, was given to CD73 mice to prevent them from developing the extremely fatal immunopathology. These results are at odds with our earlier research, which shown that adenosine's purine-source capacity—rather than its signaling role—is necessary for T. gondii survival during chronic infection. This shows that adenosine and T. gondii have a complicated interaction, but that adenosine can protect the host both during the acute and chronic stages of infection. Adenosine signaling is necessary to control inflammation and prevent collateral tissue damage during the acute stage of infection, and its decrease after the acute stage of infection can prevent the parasite from producing long-lasting cysts. et al., 2015; Mahmoud D.A.

It's intriguing how different T. gondii infection routes affect how an infection manifests in CD73 mice. These mice are extremely vulnerable to intraperitoneal infection but resistant to the acute stage of oral infection. Comparing several mice strains may reveal similar outcomes, indicating that host genetic variables may have an impact on the course of illness. For instance, C57BL/6 mice are extremely sensitive to oral infection but somewhat resistant to intraperitoneal infection, whereas BALB/c mice are resistant to both oral and intraperitoneal infection. Additionally, even when an infection is spread through the same genetic background, the sort of response that develops depends on the local immune system's cell makeup, which has the potential to polarize the response into a Th1, Th2, or even regulatory phenotype. While other compartments, such as the gastrointestinal (GI) tract

or the central nervous system (CNS) during chronic infection, may have multiple or redundant mechanisms to control immunopathology, it is likely that the regulation of the immune response in the peritoneal cavity requires the presence of adenosine to limit pathology. Notably, the peritoneal cavity lacks the additional barrier to leukocyte hyperinfiltration that the blood-brain barrier provides, which prevents neutrophils from collecting in the CNS [Mahamed D.A. et al, 2015].

Interferon gamma promotes other protective factors to restrict T. gondii replication in addition to the nitric oxide produced by inducible nitric oxide synthase (iNOS). Treatment with IFN- causes an increase of IRG (IFN-inducible GTPase) proteins near the parasitophorous vacuole in infected mouse cells, which results in membrane breakdown and parasite eradication. L-tryptophan is degraded in human cells by the enzyme indoleamine 2,3-dioxygenase (IDO), which has been found to inhibit the growth of T. gondii in a variety of types, including macrophages, astrocytes, epithelial cells, and fibroblasts. It is believed that IDO activity in the CNS is more significant than nitric oxide synthesis by IFN-induced iNOS in human host cells. Determining the function of adenosine receptor signaling in IDO-mediated regulation of T. gondii infection in human macrophages or fibroblasts would therefore be intriguing. The generation of reactive oxygen species is yet another mechanism that may cross paths with the extracellular adenosine-T. gondii interaction (ROS). It is possible that levels of ATP or its metabolites ADP and AMP are increased in CD73 mice, so it would be instructive to test the role of this pathway in the regulation of T. gondii cyst formation in CD73-deficient hosts [Mahamed D.A. et al, 2015]. Extracellular ATP can induce ROS production in T. gondii-infected macrophages.

iv. Intestinal immunopathology

The ectonucleotidase CD73 modulates inflammatory responses by successively depleting the extracellular ATP pool and releasing immunosuppressive adenosine. This regulation is probably crucial in the gastrointestinal system, where commensal bacteria in particular emit considerable amounts of ATP. Therefore, the objective of this work was to determine how the adenosinergic modulation of mice's intestines contributed to both steady-state settings and an acute infection with Toxoplasma gondii. In the gut of untrained mice, both regulatory (T-reg) and conventional (Tconv) CD4+ T cells express CD73. Adenosine production was compromised as a result of the acute T. gondii infection's downregulation of CD73 expression. The activation of adenosine receptors may be a useful strategy to control intestinal inflammation associated with decreased ectonucleotidase expression, according to an interesting finding that the expression of adenosine receptors was maintained and treatment with receptor agonists reduced immunopathology and dysbiosis [Oldenhove G. et al, 2015]. Ecto-5’-nucleotidase (CD73), which serves as the final enzyme in the production of extracellular adenosine, is highly expressed on the apical surface of intestinal epithelial cells (IECs). Adenosine signaling in IECs has been shown to have several tissue-protective effects during inflammation, but its apical expression has not been explained by prior research. The highly polarized expression of CD73 suggests that extracellular adenosine plays a significant role in mediating host-microbe interactions. The related purine metabolites 5’-AMP, inosine, and hypoxanthine do not share adenosine's bacteriostatic action against Salmonella enterica serovar Typhimurium. When compared to controls, an analysis of Salmonella colonization in IEC-specific CD73 deletion mice showed a nearly 10-fold increase in colonization [Kominsky D.J. et al, 2017]. Mice were resistant to Salmonella colitis and had decreased Salmonella loads in viscera despite increased luminal colonization by the pathogen, indicating that adenosine facilitates dispersion. Salmonella's intraepithelial localization, replication, and transepithelial translocation were severely impaired when CD73 expression was knocked down in cultured IECs. We conclude by outlining adenosine's unique antibacterial activity in the gastrointestinal system and highlighting its significant function as a regulator of host-microbe interactions. These results have extensive ramifications for the creation of novel anti-infective therapeutics [Kominsky D.J. et al., 2017].

The dual functions of the CD39/CD73 pathway in the in vivo regulation of inflammation have sparked growing interest in this system. In fact, ATP is hydrolyzed by the ectoenzymes to produce adenosine, which changes a

pro-inflammatory stimulus into an anti-inflammatory mediator. Injured, dying, or active mammalian cells, degranulated platelets, or bacteria can all produce ATP. More recently, lysosomal exocytosis dependent on caspase and pannexin 1 channels has been identified as an active mechanism of ATP secretion. Extracellular ATP binds to trimeric ion channel P2X receptors, including P2X7, an activator of NLRP3 inflammasomes, and G-protein-coupled P2Y receptors [Oldenhove G. et al., 2015]. As a chemotactic signal for neutrophils, macrophages, and immature dendritic cells, ATP is an extracellular inflammatory mediator. The activation of mast cells by ATP through P2X7 results in the generation of inflammatory cytokines, chemokines, and leukotrienes, which draw neutrophils and intensify the inflammatory response. This process has been described as a positive feedback loop in the digestive system. In addition, Oldenhove G. et al. (2015) noted that ATP functions as a costimulatory signal for T cells, controlling the growth of helper and regulatory T lymphocytes.

The membrane-bound ectonucleotidases CD39 and CD73 are necessary for the conversion of extracellular ATP to adenosine, which both directly and indirectly blocks the inflammatory effects of ATP by activating certain receptors and reducing the quantity of extracellular ATP. Four G-coupled receptors that adenosine activates either cause the intracellular levels of cyclic AMP to rise (A2A and A2B) or fall (A1 and A3). Adenosine exerts its immunosuppressive effects primarily through the A2A and A2B receptors, and it has numerous functions that try to preserve tissue homeostasis. Most immune cells as well as endothelial and epithelial cells exhibit the high-affinity receptor A2A and the low-affinity A2B receptor. Adenosine appears to decrease both innate and adaptive responses, according to a few research. Adenosine has been found to increase the immunosuppressive effect of regulatory T cells while downregulating the function of antigen-presenting cells, T helper cells, and cytotoxic T lymphocytes. In 2015, [Oldenhove G. et al.] It has been established that extracellular adenosine plays a crucial role in controlling inflammation in the intestinal mucosa. It has been demonstrated that adenosine's tissue-protective properties are mediated by signaling through particular adenosine receptors that are expressed on intestinal epithelial cells (IECs), which suppresses the production of proinflammatory cytokines and stimulates the production of cytokines that encourage the resolution of inflammation. Through the successive actions of CD39 and CD73, extracellular ATP is converted into extracellular adenosine. Although CD73 is extensively expressed on many different cell types, the colonic intestinal epithelium has the highest level of expression of any other tissue. Additionally, it is virtually entirely expressed on the apical surface of IECs. According to Kominsky D.J. et al. (2017), this highly polarized expression is particularly suggestive of a unique role for extracellular adenosine in the intestinal lumen.

Acute T. gondii significantly reduced CD73 expression, which accounts for 50% of the adenosine detected in serum, indicating that transcriptional regulation is responsible. Adenosine's importance as an anti-inflammatory agent in the digestive system was highlighted by the fact that the administration of A2A/A2B receptor agonists infected mice reduced immunological pathology and mucosal damage. These findings are consistent with a study that found that activating the A2A adenosine receptor reduced intestinal mucosal inflammation in spontaneous mouse ileitis and rabbit colitis models. Alanyl-glutamine, which promotes intestinal healing, and an adenosine A2A receptor agonist were used in combination therapy to successfully reverse enteritis brought on by Clostridium difficile toxin-A. Yegutkin et al. showed increased adenosine producing capacity in resistant C57BL/6 mice in a mouse model of Lyme illness, which were associated with reduced joint swelling. Adenosine (or adenosine receptor activation) may exert its protective effects through a variety of methods, including direct suppression of effector cells via IFN-A-dependent IL-10 activation or intracellular cAMP buildup, by raising the quantity of T-regs and boosting their immunoregulatory activity, indirect suppression of inflammation, engagement in tissue repair. Adenosine has also been demonstrated to strengthen the intestinal barrier, a result that [Oldenhove G. et al., 2015] suggest may be crucial for preventing intestinal inflammation during T. gondii infection.

TGF-part beta's in immunoregulation has a long history of research. It has been demonstrated that TGF- promotes thymic T-reg suppression and peripheral T-reg development. In more recent research, Regateiro et al. found that TGF- increased the production of CD73 mRNA in CD4+ T cells, dendritic cells, and macrophages in

vitro, excluding the predominance of opposing pro-inflammatory cytokines. Therefore, the disintegration of the T-reg cell population, the highly polarized Th1 immune response marked by high levels of IFN-, and the decreased production of TGF- may all contribute to the downregulation of CD73 expression in T. gondii-infected mice. IFN- was the most effective inflammatory cytokine at inhibiting TGF-'s in vitro activation of CD73. Likewise, the unchanged CD73 expression by CD4+ T cells from the LP in STAT-1-deficient animals during acute infection strongly supports a function for IFN-, as shown by our data. Additionally, our findings that exogenous TGF- dramatically elevated the expression of CD73 on spleen CD4+ T cells from infected mice showed indirectly that TGF- had a role in CD73 downregulation. Further research is necessary to determine whether TGF-production is affected by T. gondii infection in the stomach [Oldenhove G. et al, 2015]. The intestinal epithelium uses a variety of strategies to maintain host-microbe balance, including the creation of mucin, the secretion of antimicrobial peptides, the activation of innate and adaptive immunological responses, and the construction of tight junctions. This study suggests that the intestinal epithelium's production of extracellular adenosine, which is caused by the enzyme ecto-5'-nucleotidase (CD73), is a significant component that affects various aspects of infection by the enteric pathogen Salmonella enterica serovar Typhimurium. [D.J. Kominsky et al., 2017]

Adenosine may be harmful to the host in addition to its advantageous function as an anti-inflammatory mediator by promoting T. gondii differentiation into long-lived tissue cysts in the central nervous system. In spite of equal levels of parasite dissemination in the brain during the acute stage of infection and similar levels of leukocyte infiltration during the chronic stage of infection, Mahamed et al. discovered that CD73KO mice were less vulnerable to Toxoplasmosis. The scientists also demonstrated that CD73 expression was necessary for cyst formation and that exogenous adenosine may restore T. gondii cyst formation in host cells with defective CD73. The fact that adenosine was transported across cell membranes without the use of its receptors is intriguing and supports the positive effects of adenosine receptor agonists [Oldenhove G. et al., 2015]. Numerous interactions originating from the intestinal microbiota have been found to have a substantial impact on the physiologies of both the host and the bacteria. Lipopolysaccharide, short-chain fatty acids, toxins, and amino acid metabolites are just a few examples of the microbe-derived elements that have been proven to modify host physiology and hence contribute to both digestive health and disease. The capacity of intestinal flora to colonize and, in the case of enteric pathogens, to infect the host, is also influenced by variables that are derived from the host. At the point where the host and the intestinal microbiota meet, the intestinal epithelium occupies a special position. Therefore, mediating interactions between the host and commensal and pathogenic bacteria in the gastrointestinal lumen is a vital role of the intestinal epithelium [Kominsky D.J. et al, 2017]. It is widely known that T. gondii infection is accompanied by immunopathology. It has been demonstrated that bacteria can go from the gut to the liver, spleen, and mesenteric LN. The series of events leading to dysbiosis, which is characterized by unchecked growth of bacteria from the Enterobacteriacae family, has been pinpointed by Raetz et al. As a result of the pathogen inducing mucosal-polarized Th1-type response through T cell-intrinsic MyD88 signaling, Paneth cells were destroyed, lysozyme and defensin expression was lost, and intestinal bacteria—particularly those belonging to the Enterobacteriacae family—grew. It should be noted that T. gondii infection in germ-free animals did not result in intestinal inflammation or Paneth cell loss, supporting the pathology's function for dysbiosis. Another study linked T-cell immunity that is specific to commensal bacteria to intestinal barrier breakdown. On agonist administration, the expansion of Enterobacteriaceae in the lumen was decreased from a more than 5,000-fold expansion to a 300-fold expansion, according to our own observations [Oldenhove G. et al., 2015].

Extracellular adenosine's function in enteropathogenic Escherichia coli (EPEC) infection was previously studied by Crane et al. These findings showed that extracellular ATP is generated and then converted to adenosine during infection by several intestinal pathogens. Adenosine also appeared to change the pattern of virulence factor expression and to promote EPEC development. Together, the results of those investigations revealed that the bacteria during an infection employ adenosine and its metabolites as signals. It's interesting to note that a lot of ATP is released when S. Typhimurium is infected, just like when EPEC is infected. However, according to Kominsky D.J. et al. (2017), extracellular adenosine's function in the intestinal lumen has not been studied in relation to

Salmonella infection. IEC-derived CD73 plays a unique role in host-pathogen interactions during Salmonella infection. Salmonella growth is potently and dose-dependently inhibited by adenosine, a byproduct of CD73 activation. Next, we show that Salmonella's in vitro transepithelial translocation and intracellular replication depend on CD73 expression. The conditional knockout (KO) of CD73 in IECs, which lowers luminal adenosine levels, causes an increase in Salmonella colonization of the intestinal lumen in murine Salmonella colitis, and we demonstrate that CD73 expression is crucial for the systemic spread of Salmonella in vivo. These results strongly suggest that CD73 is essential for the control of Salmonella infection and point to adenosine as a key host-derived mediator of host-microbe interactions [Kominsky D.J. et al, 2017].

v. Human immunodeficiency virus (HIV)

Chronic immune activation and reduced T-lymphocyte activities are characteristics of human immunodeficiency virus type 1 (HIV-1) infection. Here, we find that CD73 is only weakly expressed by CD8+ T cells of HIV-infected individuals and only partially recovered following successful antiviral therapy. CD73 is both a coactivator molecule of T cells and an immunosuppressive ecto-enzyme through adenosine synthesis. Ex vivo and in vitro cell activation are inversely correlated with CD73 expression on CD8+ T cells. However, despite persistent immunological activation, CD8+ T lymphocytes from HIV controllers (HICs), which naturally regulate HIV replication, express CD73 robustly. Finally, we show that CD73 contributes to the proliferation of CD8+ T cells with specificity for HIV. Thus, we demonstrate that CD73 is essential for HIV-specific CD8+ T cell activity and that the maintenance of HIV-specific CD73+CD8+ T cells is a property of HICs. These findings point to an unique mechanism that regulates viral replication [Carrière M., et al., 2014]. On CD8 cells of HIV elite controllers, a molecule involved in regulating T-cell activation and activity is substantially expressed. T-cell surface molecule CD73 is involved in a number of processes, including the hydrolysis of extracellular adenosine monophosphate into adenosine, which prevents the proliferation of T cells. Low amounts of CD73, which may modulate T-cell activity and activation, were discovered on the T cells of HIV-positive patients in earlier investigations. The role of CD73 in HIV-infected elite controllers, or people who maintain undetectable HIV RNA levels without receiving antiretroviral medication, has now been studied by French researchers (ART) RT Gandhi (2014)

Rapid immunological activation, the growth of CD8+ T cells specific for HIV, and a corresponding decline in plasma viremia are all features of the acute phase of human immunodeficiency virus (HIV) infection. Chronic infection results in impaired CD8+ T-cell function and intense viral multiplication. This lack of viral control is partly a result of the immune system becoming worn down, which is a side effect of both large CD4 depletion and systemic immunological activity. In the gut, where the majority of the body's immune cells are found, viral replication related to the loss of immunological control also takes place. A very small proportion of untreated HIV-positive people (less than 1%) develop a state of apparent lasting control of HIV replication, known as HIV controllers (HICs). The CD8+ T-cell response appears to be crucial for keeping HIV under control in HICs. In many of these patients, HIV antigen-stimulated CD8+ T cells can multiply, generate perforin and a variety of cytokines, and can block viral replication when cocultured with infected CD4+ T cells. A recent genome-wide association analysis indicated that the main genetic factors influencing the shape of class I antigen-presenting molecules are those related to the natural regulation of HIV. These findings suggest CD8 T cells are involved in the suppression of HIV, but the precise mechanism is still unknown because these genotypes are not exclusive to HICs [Carrière M., et al., 2014].

A cell surface protein called CD73, which is expressed by immunological and endothelial cells, hydrolyzes extracellular nucleoside monophosphates like adenosine monophosphate (AMP) into adenosine. Adenosine reduces the generation of cytokines and the cytotoxicity of activated T cells, as well as T-cell proliferation. Additionally, CD73 might facilitate lymphocyte adherence to endothelial cells. It is also a costimulatory molecule that sends T cells strong activation signals. As a result, CD73 might play two different parts in the regulation of T-cell immunological responses. The endogenous GPI phospholipase D (GPI-PLD), which is responsible for the

cleavage and release of several GPI-anchored membrane receptors, controls CD73 expression, which is consistent with a complicated role. The GPI-PLD is active in the intracellular compartment but not in the circulation or extracellular fluids, while being prevalent in the serum [Carrière M., et al, 2014]. Blood samples from 16 HIV-infected patients who were not receiving ART, 16 HIV-infected patients who were receiving suppressive ART, and 7 elite controllers were examined for CD73 expression on CD8 cells. Compared to HIV-infected individuals on suppressive ART, patients who were not receiving antiretroviral therapy (ART) exhibited lower blood levels of CD73+ CD8 cells (6.9 percent vs. 37.0 percent). CD73+ CD8 cell counts were highest in elite controllers (61.4 percent). There was an unfavorable relationship between CD73 and CD38 expression on memory CD8 cells in HIV-infected individuals who were not elite controllers. RT Gandhi (2014)

It had been noted that CD8+ T-cells' surface 5-ecto-nucleotidase activity was lower in HIV-infected patients than in controls before CD73 was identified. Recently, it has been demonstrated that the downregulation of CD73 on CD8+ T cells is associated with immune activation and causes functional deficits in HIV infection. This is because sorted CD73+CD8+ T cells produce more cytokines and have a higher capacity for proliferating after being stimulated with HIV than CD73-CD8+ T cells. However, the specific function of CD73 and how it affects CD8+ T-cell performance during HIV infection are not yet fully understood. Here, we compared CD73 expression on peripheral blood mononuclear cells (PBMCs) and gut biopsies from various groups of HIV-infected patients and healthy volunteers, examined the correlations with clinic and immune activation parameters, and then centered on the impact of in vitro downregulation of CD73 using either anti-CD73 antibody or siRNA on CD8 T-cell response. Our initial research indicates that CD73 is essential for HIV-specific CD8+ T cell function and that the maintenance of an HIV-specific CD73+CD8+ T cell population is a feature of HICs [Carrière M., et al., 2014].

We find that immunological activation is correlated with downregulation of CD73+CD8+ T cells in HIV-infected individuals, both in situ and in the circulation. The observation of a considerable decrease in 5'-nucleotidase activity in rat lymphocytes following concanavalin A stimulation is consistent with the relationship between CD73 expression and the degree of cell activation. It is also in line with research on CD4+ T cells, which demonstrates that during HIV infection, CD4+CD73+ T-cell frequency and number are lowered and inversely linked with CD4+CD38+DR+. While this work was being completed, another team published results that were quite similar by examining the CD73 expression on several T-cell subsets from HIV patients. Using a functional experiment based on in vitro-mediated CD73 downregulation, they were unable to demonstrate the functional impact of CD73 deficiency, but [Carrière M., et al, 2014].

The development of CD8+ T lymphocytes that are specific for an antigen is aided by CD73. This is consistent with the finding that in patients with primary immunoglobulin deficiency, there is a substantial association between in vitro proliferative abilities in response to mitogens and the quantity of CD73 molecules per CD73+CD8+ cell. Additionally, CD73 can prevent TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis, and during the acute phase of HICs, when the viral load is found measurable in HICs, the concentration of plasma-soluble TRAIL increases (for approximately 6 months after primary HIV-1 infection). Then, we propose that CD73 may be crucial for the development of a potent anti-HIV memory CD8+ T-cell response during the acute phase in HICs, which would help to control viral infections other than HIV. (M. Carrière et al., 2014)

We found no association between HLA-B27 (or -B57) genotypes and CD73 expression in HICs. These genotypes are the main genetic determinants associated with the natural control of HIV (data not shown). It has been demonstrated that HICs may better control various chronic infections, including HCV infection, and this was true regardless of HLA-B27 or -B57. However, not all HICs had this phenotype. These findings imply that additional mechanisms to restricted HLA may be involved in the broad protection of HICs against viral infections. (M. Carrière et al., 2014)

On the other hand, it has long been known that CD73, which is produced by both tumor and host immune cells, is damaging in the context of cancer. According to the postulated mechanism, CD73 suppresses antitumor-

specific T-cell activity by producing adenosine in the antitumor microenvironment. However, we argue that the balance is pushed toward coactivation and away from CD73's enzymatic activity in the context of widespread immunological activation and inflammation. We recently demonstrated that regulatory T cells/CD39+ cells inhibit the expression of IL-2 by activated CD4+ T cells in a medium supplemented with adenosine triphosphate, suggesting that this balance is presumably dependent on cell type and concentration of purinergic molecules in the microenvironment [Carrière M., et al, 2014]. The chicken-and-egg dilemma makes it difficult to determine whether the high levels of CD73+ CD8 cells present in these individuals are a cause or an effect of their patients' ability to regulate their virology. However, the higher our chances are at creating therapies that could cause this situation, the more we comprehend about the immunologic traits of top controllers [Gandhi R.T., 2014].

vi. Ecto-5′-Nucleotidase (CD73) reduces Mortality and Organ Injury in Sepsis

Adenosine receptors control the host's response to sepsis, and extracellular adenosine concentrations rise during sepsis. In this study, we looked into how the ecto-5′-nucleotidase (CD73), an ectoenzyme that produces adenosine, controls immunological and organ function during sepsis. By ligating and puncturing the cecums of CD73 knockout (KO) and wild type (WT) mice, polymicrobial sepsis was generated. Compared to WT mice, CD73 KO mice displayed higher death rates, which were linked to higher bacterial counts and greater levels of inflammatory cytokines and chemokines in the blood and peritoneum. Increased myeloperoxidase activity, neutrophil infiltration, and raised pulmonary cytokine levels were all signs that CD73 loss accelerated lung injury [Haskó G. et al, 2011]. As shown by increased caspase-3 and poly(ADP-ribose) polymerase cleavage and enhanced NF-B activation, CD73 KO mice experienced higher apoptosis in the thymus. Higher blood urea nitrogen and higher cytokine levels in the kidney of septic CD73 KO mice suggested greater renal impairment. Greater p38 MAPK activation and lower Akt phosphorylation were linked to increased renal damage in CD73 KO mice. Using, -methylene ADP to pharmacologically inactivate CD73 de WT mice increased cytokine levels in the blood and peritoneal lavage fluid. These results imply that adenosine generated from CD73 may be helpful in sepsis. [Gábor Haskó et al., 2011]

When microbial invasion causes systemic disease, sepsis results. Even with improvements in contemporary hemodynamic, antimicrobial, and ventilatory clinical support, sepsis remains a serious clinical issue without a cure. According to Haskó G. et al. (2011), sepsis alone causes 215,000 deaths in the United States each year. Although the etiology of multiorgan harm brought on by sepsis that results in death is not fully known, current research suggests that an early hyperinflammatory process and a subsequent immune paralysis are responsible for sepsis-related mortality and morbidity. In the early stages of sepsis, there is an initial hyperinflammatory response that is accompanied by unchecked, excessive cytokine synthesis. This excessive cytokine production can damage a variety of tissues and result in organ damage and malfunction. An immunological paralytic phase follows this hyperinflammatory phase, and the spleen, kidney, liver, and heart all exhibit increased apoptotic cell death [Haskó G. et al., 2011].

By interacting with one or more of the four G protein-coupled adenosine receptors, the physiologically active extracellular signaling molecule adenosine controls a wide range of immunological activities (A1, A2A, A2B, and A3). Since sepsis is linked to various metabolically demanding circumstances, such as inflammation, hypoxia, ischemia, or trauma, systemic adenosine levels are elevated in sepsis- and septic shock-infected mice and human patients [Haskó G. et al., 2011]. Adenosine receptors' ability to control the host's response to sepsis is becoming more and more clear. Recent research has shown that the A1, A2B, and A3 receptors reduce mortality, inflammation, renal failure, and liver injury in the mouse cecal ligation and puncture (CLP) model of polymicrobial sepsis, a model that is clinically relevant. Contrarily, we demonstrated that A2A receptor activation enhanced splenic apoptosis, decreased bacterial clearance, and low levels of inflammatory cytokines, which all contributed

to sepsis' deadly effect. These findings support the idea that distinct adenosine receptors can have various, often conflicting impacts on immunity during sepsis, and that extracellular adenosine is a critical regulator of immunological processes in sepsis-susceptible mice.

Release of precursor adenine nucleotides, primarily ATP, from the cell followed by extracellular catabolism to adenosine by a cascade of ectonucleotidases, including CD39 (nucleoside triphosphate diphosphorylase) and CD73 (ecto-5'-nucleotidase), is one important pathway contributing to increased extracellular adenosine levels during metabolic stress. A transmembrane molecule called CD39 catalyzes the conversion of ATP and ADP to AMP, which starts the production of extracellular adenosine. The dephosphorylation of AMP to adenosine is catalyzed by CD73, a 70-kDa glycosyl phosphatidylinositol-anchored cell surface protein having ecto-5'-nucleotidase enzyme activity. As a result, by catalyzing the final stage of the ATP breakdown cascade, this enzyme plays a crucial part in the production of extracellular adenosine [Haskó G. et al, 2011]. In fact, it has been suggested that CD73 is the enzyme that restricts the rate of adenosine synthesis under metabolic stress. Numerous in vivo experimental models have clearly demonstrated how CD73 regulates the immune system. Methotrexate's ability to reduce inflammation has been said to be reliant on CD73's capacity to make adenosine. Adenosine produced by CD73 reduces the trafficking of polymorphonuclear neutrophils (PMNs) during acute lung damage brought on by LPS. Similar to how CD73-derived adenosine guards against acute lung injury brought on by bleomycin and ventilation. Extracellular adenosine produced during hypoxia is a strong anti-inflammatory signal for PMNs in vivo, as shown by the requirement of CD73 activity to inhibit vascular leak and neutrophil infiltration into different organs in hypoxia models. In renal and cardiac ischemia, CD73-generated adenosine has also been demonstrated to play a protective effect. [Gábor Haskó et al., 2011]

Although the specific adenosine receptors' roles in sepsis have been discussed, it is still unclear how CD73-derived adenosine interacts with the other three adenosine receptors. This work examined the effects of targeted genetic deletion or pharmacological inactivation of the adenosine-producing CD73 on the progression of the host's response to sepsis using the CLP model [Haskó G. et al., 2011].

According to this study, CD73 prolongs septic mouse survival and is associated with reduced bacterial growth, as well as reduced lung and kidney damage [Haskó G. et al., 2011].

Combined with evidence from the literature, our discovery that CD73 increased the lifespan of septic mice suggests that elevated adenosine levels and adenosine signaling can reduce sepsis-related mortality. Previous research has demonstrated that improving survival in sepsis by interfering with adenosine metabolism downstream of CD73 increases extracellular adenosine levels. In models of sepsis, inhibiting adenosine deaminase, an enzyme that converts adenosine to its less active metabolite inosine, reduced inflammation and microvascular dysfunction while increasing survival. Additionally, pharmacological suppression of the enzyme adenosine kinase, which lowers extracellular adenosine levels by catalyzing the phosphorylation of adenosine to nucleotides, boosted survival in sepsis. The tissue-protective properties of CD73 in our sepsis model are linked to its enzymatic activity and role in raising extracellular adenosine levels, according to our reconstitution studies with NECA. It is particularly interesting because prior research employing adenosine receptor-specific knockout mice shown that endogenous adenosine enhances survival in sepsis by activating A1, A2B, and A3 receptors. Despite the fact that endogenous adenosine's activation of the A2A receptor reduces survival, our data with CD73 inhibition and earlier findings with adenosine deaminase and kinase inhibition imply that the advantageous effects of adenosine via A1, A2B, and A3 receptor signaling can outweigh the detrimental effect of A2A receptor signaling. Bacterial numbers are reduced when CD73 is present [Haskó G. et al., 2011]. Adenosine is often an inhibitory mediator, thus we hypothesized that adenosine produced from CD73 would also reduce antibacterial immune responses. This result was quite unexpected. The lower bacterial load in CD73-sufficient mice may have been caused by increased A1 receptor signaling that boosted superoxide generation or increased neutrophil chemotaxis via A3 receptors. In fact, according to Nakav et al., the expression of the pro-inflammatory A2A receptor is low during the beginning of peritonitis while the A1 receptor predominates. Therefore, it is

conceivable that CD73-derived adenosine enhances antibacterial defense via A1 receptors on neutrophils in the initial hours following the induction of peritonitis. Additionally, the discovery that A3 adenosine receptor WT mice showed lower aerobic bacterial counts after CLP-induced sepsis as compared to their KO counterparts supports a function for A3 receptors in regulating bacteria [Haskó G. et al, 2011].

Evidence supports the idea that activated neutrophil infiltration into immune-competent organs after infections causes tissue damage. Our results showed that in CD73-sufficient animals, fewer neutrophils were recruited into the lung and spleen, supporting this theory. This could indicate a direct role for adenosine in influencing neutrophil activation and transmigration [Haskó G. et al., 2011] or it could be the effect of lower neutrophil activation due to the suppressed cytokine storm.

Our findings that sepsis-induced cytokine storms are reduced in the presence of CD73 may shed more light on the defense mechanisms of CD73-derived adenosine in sepsis. In studies resembling ours, it was found that CD73 inhibits the generation of proinflammatory cytokines in murine gastritis, cardiac allograft vasculopathy, and intestinal ischemia-reperfusion injury. In crucial organs including the lung and kidney of septic mice, the tissue-protective effects of CD73 were strongly associated with the declines in inflammatory cytokine levels. Because sepsis causes multiorgan failure and inflammatory tissue damage, these organ-protective effects may help explain why survival in septic CD73-deficient animals is better. Sepsis also causes high death rates. The lung and kidney protection described in this work is consistent with other research demonstrating that CD73 prevents lung injury caused by bleomycin, ventilators, and LPS as well as kidney injury caused by ischemia-reperfusion [Haskó G. et al, 2011].

Sepsis causes parenchymal tissue destruction and organ injury through a variety of methods. For instance, it is well known that enhanced NF-B and p38 activation, as well as the inactivation of Akt, can cause tissue damage and cell death, which might compromise clinical outcome in sepsis. An essential intracellular route via which CD73 and adenosine result in reduced organ harm in sepsis may be represented by the overexpression of Akt (kidney) activation and the upregulation of p38 (kidney) and NF-B (thymus) activation [Haskó G. et al., 2011]. The finding that disrupting adenosine signaling by systemic administration of the adenosine receptor antagonist aminophylline led to higher p38 and lower Akt activation in anoxia-induced brain confirms an important role for adenosine signaling in decreasing p38 and increasing Akt activation in tissue stress. Sepsis can cause severe apoptosis in immune tissues like the thymus and the spleen in addition to harming nonimmune organs. It has been suggested that the thymus contains higher levels of caspase-3 activity than other caspases and that this activity is crucial for the death of thymocytes brought on by sepsis. Our findings that septic CD73 WT animals had lower amounts of caspase-3 cleavage and PARP cleavage in the thymus suggest that this enzyme and its byproduct, adenosine, are crucial inhibitors of apoptotic events. In conclusion, this research shows that CD73 is a crucial control point for modulating the host's reaction to sepsis [Haskó G. et al., 2011].

3. Functions in the Urinary system: NEPHROLOGY

1. ROLE of CD73 IN NEPHROLOGY

1. Cluster of differentiation 73 (CD73) and the renal functions

1. The impact of CD3

We proposed that hypoxia-inducible CD73-dependent adenosine production may be crucial during renal IP based on earlier research that demonstrated tissue protection by extracellular adenosine. Therefore, using a previously

published in situ IP model that consists of four cycles of intermittent renal artery occlusion and reperfusion, we first examined renal CD73 expression and function. For isolated renal artery occlusion, intermittent renal artery occlusion was carried out utilizing a hanging-weight technique. The kidney tissue of unpreconditioned, age-, weight-, and gender-matched littermates was contrasted with that from mice that had undergone IP. Renal tissue was collected for real-time reverse transcriptase-PCR analysis at the relevant time points following IP therapy in order to define the transcriptional effects of IP. The expression of CD73 mRNA is strongly induced. CD73 protein induction following IP was validated by Western blot analysis, immunohistologic staining, and imaging with confocal laser scanning microscopy. We assessed the activity of the ecto-5'-nucleotidase enzyme to evaluate the functional induction of CD73. After 30 minutes of IP therapy, ecto-5'-nucleotidase activity was almost three times higher than it was before. Together, our findings clearly support renal IP-induced CD73 upregulation [Grenz A. et al., 2007]. By activating the adenosine A2A receptors (A2ARs) on neutrophils and macrophages, CD73 prevents inflammatory damage and necrosis during renal ischemia-reperfusion injury in the proximal tubular epithelial cells of the kidney. In the context of diabetic nephropathy, CD73 is protective due to its epithelial and endothelial activities. Endothelin-1 and A2BR are required for CD73 to increase hypertension in chronic renal disease. Angiotensin II, or AngII (Minor M. et al., 2019). In addition to acute kidney injury, diabetic nephropathy, a chronic kidney condition that can arise in people with long-term diabetes mellitus, also has a protective role for CD73. Streptozotocin (STZ), a chemotherapy drug, causes insulin-producing pancreatic cells to be selectively destroyed in mice. This results in a diabetic state and nephrotoxicity, simulating some elements of diabetic nephropathy. In WT mice given a 16-week course of STZ, the amount of adenosine in the kidneys was increased by two times while remaining unaltered in Nt5e mice. Increases in drinking volume, urine output, glomerular filtration rate, and albuminuria were all symptoms of more severe renal failure in Nt5e mice. Mice lacking endothelium A2BR displayed similar outcomes. The administration of soluble CD73 reversed all renal damage markers and returned renal adenosine content to WT values. Therefore, in a mouse model of diabetic nephropathy, the controlled synthesis of extracellular adenosine via CD73 and subsequent activation of the endothelium A2BR are important protective mechanisms. Adenosine administration is protective in the rat, much like it is in mice, and increased CD73 activity appears to be an early event in STZ-mediated renal damage [Minor M. et al, 2019].

Renal ischemia preconditioning (IP) induces CD73 [Grenz A. et al., 2007]:

- ☐ Renal IP model in mice. Age, gender, and weight-matched mice were given a right nephrectomy, then in situ IP using a hanging-weight method to occlude the left renal artery without damage. The IP protocol included four cycles of ischemia/reperfusion and the corresponding reperfusion periods.
- ☐ IP causes CD73 mRNA to be produced. Kidneys were removed at the specified dates, total RNA was extracted, and real-time reverse transcriptase-PCR was used to assess CD73 mRNA levels. Data are expressed as a fold change in comparison to the control and were calculated relative to -actin.
- ☐ IP Western blotting causes the CD73 protein to be produced. Proteins were separated by SDS-PAGE and transferred to nitrocellulose after kidneys were removed at the appropriate times, flash-frozen, and lysed. Anti-CD73 antibody was used to probe membranes. A sample of three experiments is displayed. As a check for protein loading, the same blot was once again probed for -actin.
- ☐ IP immunohistochemistry induces the expression of CD73 protein. Mice of the wild type (WT) were given IP. After the specified durations, kidneys were removed, cut into sections, stained with a CD73 antibody, and observed using confocal laser scanning microscopy. The control was tissue from a perfused but unconditioned WT mouse. Top left of the image is "negative" staining of WT tissue using only a secondary antibody.
- ☐ IP stimulates the activity of the CD73 enzyme. After IP therapy, kidneys were removed, flash-frozen, and extracts were made according to the instructions in the Materials and Methods section. By measuring the conversion of CIMP to cinosine in the presence and absence of 5'-(methylene) diphosphate, 5'-nucleotidase enzyme activity was determined (APCP). According to Grenz A. et al. (2007), enzyme activity is reported as nmol/h per mg protein of APCP-inhibitable IMP hydrolyzing activity.

A commercially available colorimetric technique was used to quantify the levels of plasma and urine creatinine 24 hours after renal ischemia. Using a flame-emission photometer, the amounts of Na+ and K+ in the plasma and urine were measured. Using established formulas, renal excretory and hemodynamic data were computed. After 24 hours, kidneys were removed and kept at 80°C for later study [Minor M. et al., 2019].

In addition to TNAP, CD73-produced adenosine also regulates renal vascular function. The kidneys have the highest enzymatic activity of all the body's tissues for CD73. Enzyme histochemistry and immunostaining studies on the proximal tubular cells, the peritubular space, intercalated cells of the distal nephron, and cortical fibroblasts in rat and mouse kidneys demonstrated expression of CD73. Depending on the type of kidney injury, CD73 has a mixed protective and injury-promoting effect in the kidney [Minor M. et al., 2019].

I/R is a major factor in acute kidney injury, and CD73 is essential for protection in this situation. The lower cortex and outer medulla of the kidney experience the greatest degree of damage during I/R, and these areas also have the highest levels of CD73 expression. Using mechanistic in vivo research with Nt5e mice and animals with a targeted deletion of CD73 from the proximal tubule compartment, it was demonstrated that CD73 expressed on proximal tubular epithelial cells is a crucial mediator of the protective response. In fact, a Foxd1-driven Cre promoter's targeted deletion of CD73 from cortical fibroblasts led to a more time-dependent damage response [Minor M. et al., 2019]. Therefore, following I/R injury, CD73 on cortical fibroblasts and proximal tubular epithelial cells confers a protective effect. Conversely, there was no discernible difference in the severity of renal I/R injury between control animals and mice that had CD73 specifically deleted from their dendritic cells or macrophages. Injury in the Nt5e and PEPCKCreCD73f/f mice models was reversed by in vivo restoration of 5'-NT activity. Because it specifically targeted the A2AR on neutrophils and macrophages, localized synthesis of adenosine via CD73 was a crucial factor at the site of injury [Minor M. et al., 2019].

A strategy to reduce the proinflammatory effects of extracellular ATP may include CD73 activation and subsequent buffering of extracellular adenosine-ATP levels, in addition to the protective benefits of adenosine. Studies revealing that Nt5e mice experience spontaneous proteinuria and decrease in renal function as they age due to an autoimmune inflammation affecting the glomerular endothelium provide evidence of the anti-inflammatory effects [Minor M. et al, 2019]. It is possible that the situation in the kidney is significantly more complicated than what was previously stated and involves a group of enzymes that includes CD73 and TNAP but also goes beyond them. For instance, Street and colleagues show that deletion of CD73 only significantly reduces adenosine synthesis by 46% in dorsal root ganglion neurons and spinal neurons (at pH 7.4). However, adenosine synthesis is reduced by 69 percent when CD73 and prostatic acid phosphatase (PAP) are both knocked out simultaneously. Additionally, these researchers discover that adding a TNAP inhibitor to neurons deficient in both PAP and CD73 is the only way to completely suppress adenosine production. It is important to note that in the current investigation, some adenosine was produced from 5'-AMP in CD73 kidneys treated with levamisole, indicating the participation of other kidney enzymes [Jackson E.K. et al, 2014].

Angiotensin II (ANG II) was discovered to cause a two- to three-fold increase in CD73 mRNA, protein, and activity, which is correlated with a four-fold rise in renal adenosine concentration. As this effect is reduced in Nt5e animals, the increase in ANG II-stimulated renal adenosine synthesis was CD73-dependent. In WT mice but not Nt5e mice, systolic blood pressure rises after ANG II infusion. Since blood pressure is not increased in ANG II-infused animals missing the A2BR, this effect is mediated via the A2BR. It was suggested that endothelin-1 expression is encouraged via a positive feedback loop between CD73 and the A2BR with hypoxia-inducible factor-2, which in turn encourages hypertension and renal damage. The majority of in vivo investigations, however, relied on connecting variations in expression levels. Nevertheless, CD73 protein levels are two to three times greater in patients with chronic renal disease and hypertension than in those with mild illness and no hypertension, and seven times higher than in normal patients (as determined by immunohistochemical staining). Therefore, it would be crucial for future studies to evaluate the impact of CD73 on different cell types in renal damage in the presence of hypertension. In 2019, Minor M. et al.

Recent studies in brain tissue reveal that CD73 is not necessary for the conversion of 5′-AMP to adenosine because of tissue nonspecific alkaline phosphatase (TNAP), which like CD73 is a GPI-anchored ecto-enzyme with 5′-nucleotidase activity. CD73 metabolizes extracellular 5′-AMP to adenosine. We looked into whether both TNAP and CD73 were involved in the renovascular metabolism of 5′-AMP because adenosine plays a significant role in regulating renovascular function. To verify this, we used mass spectrometry to measure the levels of 5′-AMP, adenosine, and inosine (an adenosine metabolite) in the renal venous system of isolated, perfused mouse kidneys. We then looked at how 5′-AMP, which was administered intrarenally to the renal vasculature, was converted to adenosine. It takes the suppression of both CD73 and TNAP to stop the synthesis of adenosine because they work together to metabolize luminally applied 5′-AMP in the renal vasculature [Jackson E.K. et al., 2014].

The majority of organ systems depend on CD73, an ecto-5′-nucleotidase that is glycosylphosphatidylinositol (GPI)-anchored and found on the cell surface, to convert extracellular 5′-AMP to adenosine. Regarding the kidney, CD73 contributes to the adenosine production that facilitates tubuloglomerular feedback, provides renoprotective adenosine that increases the kidneys' tolerance for acute ischemia-reperfusion, and produces adenosine in response to long-term angiotensin II exposure that causes A2B receptor-induced chronic renal disease and hypertension. Surprisingly, however, preliminary research suggests that the rise in renal venous adenosine that occurs when exogenous 5′-AMP is administered into the renal artery is typical in CD73 kidneys. These results imply that alternative pathways of adenosine production may participate in the metabolism of 5′-AMP to adenosine in the luminal aspect of the renal vasculature since adenosine output measured in the renal vein after administration of 5′-AMP into the renal artery likely reflects largely renovascular metabolism of adenosine. If accurate, this is a crucial idea given the potent renovascular effects of adenosine. In 2014, Jackson E.K. et al.

The primary isoform of alkaline phosphatase expressed in the kidney, tissue nonspecific alkaline phosphatase (TNAP), is a GPI-anchored ecto-nucleotidase that can catalyze the conversion of 5′-AMP to adenosine. Zhang and colleagues' significant research shows that TNAP mediates 5′-AMP conversion to adenosine in the brains of CD73 mice but not CD73+/+ mice and that TNAP can make up for CD73 deficiency in the mouse brain. These details lead us to postulate that CD73 and TNAP both participate in the conversion of 5′-AMP to adenosine in the renal vasculature, and that inhibition or deletion of just one of either enzyme is insufficient to stop renal vascular 5′-AMP metabolism because the alternative enzyme is still active and offers a different metabolic pathway. Our goal here is to put this idea to the test [Jackson E.K. et al, 2014].

There are numerous traits that CD73 and TNAP share. Both are ecto-enzymes that can metabolize 5′-AMP to adenosine, are GPI-anchored to cell membranes with the catalytic domains facing the extracellular space, contain metal ions (such as Zn2+), are glycosylated, have similar molecular weights, form homomeric dimers, and are widely expressed in many tissues and cells. Despite these similarities, researchers have largely neglected the potential roles of TNAP in this regard and have instead concentrated almost completely on CD73 as "the" adenosine-generating enzyme [Jackson E.K. et al, 2014].

Several investigations have suggested that TNAP plays a physiological role in the synthesis of adenosine, despite the fact that the literature on this topic is lacking. For instance:

• According to a study by Ohkubo and colleagues, TNAP rather than CD73 appears to be responsible for the ecto-AMP phosphohydrolase activity of NG108-15 cells, a cell line created by crossing mouse neuroblastoma with rat glioma cells;

• Research by Zhang and colleagues shows that 5'-AMP decreases synaptic transmission in CD73+/+ and CD73 mouse brain slices identically, and that TNAP inhibition only reduces the effects of 5'-AMP in CD73 mouse brain slices;

• According to Kuzhikandathil and colleagues, the renal D1 receptor is downregulated by 3',5'-cAMP in the rat kidney by a mechanism that is only partially responsive to the TNAP inhibitor levamisole; and

• According to Pettengill et al., CD73 and TNAP play a critical role in the production of adenosine from 5'-AMP in human blood, especially in newborn blood. But it's still unclear how CD73 and TNAP compare to one another in the kidneys' synthesis of adenosine. In 2014, Jackson E.K. et al.

The function of adenosine in renal sympathetic neurotransmission can support the role of TNAP in the renal synthesis of adenosine. In this regard, our previous studies show that pharmacological inhibition of A1 receptors reduces the renovascular responses to stimulation of the renal sympathetic nerve in isolated, perfused rat kidneys; and our subsequent studies in isolated, perfused kidneys from A1 receptor null mice confirm the idea that renal sympathetic neurotransmission depends on A1 receptor signaling to provide for a full response to renal sympathetic activation. However, it turns out that CD73 is not necessary for typical renal sympathetic responses and appears to not be necessary for renal adenosine synthesis when comparing the effects of renal sympathetic nerve stimulation in CD73 vs. CD73+/+ kidneys. Overall, these findings imply that renal adenosine synthesis may involve more factors than only CD73 mediating the conversion of 5'-AMP to adenosine. In 2014, Jackson E.K. et al.

Strong evidence points to both CD73 and TNAP playing a significant and cooperative role in the renovascular metabolism of 5'-AMP to adenosine. This conclusion is supported by two main lines of evidence and reasoning. First, whether TNAP is suppressed determines if CD73 deletion changes the renovascular extraction of 5'-AMP. CD73 knockout has no impact on the renovascular extraction of 5'-AMP when TNAP is not inhibited, but knockout of CD73 decreases renovascular extraction of 5'-AMP when TNAP is blocked. Additionally, whereas TNAP inhibition decreases renovascular extraction of 5'-AMP in CD73 kidneys, blocking TNAP has no effect on it in CD73+/+ kidneys. Second, whether TNAP is suppressed determines if CD73 deletion modifies the renovascular synthesis of adenosine and inosine from 5'-AMP. The generation of adenosine/inosine from 5'-AMP in the renal vasculature is unaffected by CD73 deletion when TNAP is not inhibited, but it is decreased when TNAP is stopped. Furthermore, while TNAP inhibition decreases renovascular production of adenosine/inosine in CD73 kidneys,

blocking TNAP does not affect the formation of adenosine/inosine from 5′-AMP in CD73+/+ kidneys. Together, these results imply that when the CD73 pathway is inhibited, the TNAP pathway compensates and vice versa, and that the renovascular 5′-AMP adenosine inosine pathway is only inhibited when both CD73 and TNAP are blocked concurrently. In 2014, Jackson E.K. et al.

We first demonstrated that IP induces CD73, which contributes to elevated renal adenosine concentrations. We then looked into the functional significance of CD73 in renal protection. Before renal ischemia with or without IP, we gave mice either the specific CD73 inhibitor APCP or a vehicle to test the role of CD73 in IP-mediated renal protection. Renal CD73 enzyme activity was reduced almost twofold after APCP therapy. Because APCP is not an irreversible inhibitor of ecto-5′-nucleotidase enzyme activity and was diluted roughly 50 times during the production of the tissue extracts, the real degree of inhibition in vivo was probably much higher [Grenz A. et al, 2007]. The way CD73 and TNAP interact with ATP and ADP is an intriguing distinction between the two proteins. This is because ATP and ADP cause CD73 to experience "feed-forward" inhibition. Therefore, CD73 is blocked when cells produce ATP or ADP until ATP and ADP are primarily converted to 5′-AMP. It is known that TNAP may metabolize ATP all the way to adenosine without the assistance of CD39 (ATP ADP 5′-AMP adenosine). As a result, whether extracellular levels of ATP/ADP are high or low may affect the relative relevance of CD73 vs. TNAP in vivo, with high levels preferring the TNAP route and low levels favoring the CD73 pathway. As a result, it's possible that CD73 and TNAP's interaction roles are temporally dynamic [Jackson E.K. et al, 2014]. The existence or absence of hypoxia may also affect the significance of CD73 relative to TNAP. According to Poth and colleagues, hypoxia transcriptionally regulates the expression of CD73 because of elevated amounts of hypoxia-inducible factor-1 inside of cells. It is unknown if this holds true for TNAP. The relevance of CD73 vs. TNAP would also rely on the presence of hypoxia, which would prefer the CD73 pathway if hypoxia enhances the expression of CD73 but not TNAP [Jackson E.K. et al, 2014]. What is the mechanism by which TNAP makes up for CD73's absence in the renal vasculature (and vice versa)? It's possible that TNAP expression is elevated as a result of CD73 deletion. The renal expressions of TNAP mRNA, protein, and activity between CD73+/+ and CD73 kidneys, however, do not significantly change, suggesting that this is not the case. Another theory is that when CD73 is either knocked out or TNAP is suppressed, enough 5′-AMP accumulates behind the blocked pathway to speed up the rate of adenosine production from the alternate pathway, fully compensating for the loss of one arm of the adenosine-producing system. In the event that the alternate pathway is not saturated, full compensation would be anticipated. Given that TNAP can counteract CD73 (and vice versa) when 5′-AMP levels are high (10 mol/l), it is unlikely that the alternative routes will become saturated with 5′-AMP in vivo [Jackson E.K. et al, 2014].

We then conducted research in CD73 gene-targeted animals in light of these pharmacologic results showing that CD73 inhibition attenuates the renal protective effects of IP. after concluding that these mice's renal tissue is devoid of CD73 enzyme activity. The current study proves that neither CD73 nor TNAP are specifically required for adenosine synthesis from 5′-AMP in the milieu of the luminal surface of the renal vasculature, which is most likely made up of endothelial cells. Evidently, in this case, the absence of one enzyme can be completely made up for by the presence of the second enzyme. We don't believe that this is true for all renal microenvironments,

either. We specifically postulate that TNAP largely facilitates the conversion of 5'-AMP to adenosine in particular renal compartments whereas CD73 primarily controls the synthesis of adenosine from 5'-AMP in other renal compartments. Additionally, although both CD73 and TNAP are present in various renal microenvironments, the activity of the other enzyme does not fully make up for the loss of one activity. This may be the reason Huang et al discovered that the adenosine-dependent phenomena of tubuloglomerular feedback only attenuates, but does not eliminate, the effect of CD73 deletion [Jackson E.K. et al, 2014], is. Furthermore, following IP therapy, myeloperoxidase activity in renal tissue did not decrease in CD73 animals. Together, our findings provide the first genetic proof of CD73-dependent IP renal protection. et al., 2007; Grenz, A. It has been proven that CD73 contributes to perioperative ischemia acute kidney damage. In particular, clinically relevant quantities of the volatile anesthetic isoflurane substantially increased CD73 on proximal tubule cells via a TGF-1-dependent mechanism. Additionally, a TGF-1/CD73/A2AR-dependent pathway provided isoflurane protection against renal tubular necrosis and inflammation after I/R injury. Together, these results demonstrate the translational significance of CD73 in the defense against acute kidney injury at a molecular level. While the molecular mechanisms regulating the protective responses are tissue-specific, CD73 plays a protective role during I/R injury in the brain, heart, kidney, and liver tissues. In 2019, Minor M. et al. [Minor M. *et al*, 2019]

2. Why Adenosine?

Adenosine has long been recognized as a key mediator in the kidney, primarily via controlling glomerular filtration. Castrop et al. investigated tubuloglomerular feedback in the kidneys of Cd73j/j and wild type mice to determine the role of CD73-generated adenosine in local hemodynamic regulation mechanisms in the kidney. It is interesting to note that plasma osmolarity, plasma concentrations of Na+, Clj, BUN, creatinine, uric acid, and total protein, as well as renal blood flow, renal vascular resistance, stimulation of renin secretion by furosemide, were all shown to be normal in Cd73-deficient mice. However, compared to wild type animals, Cd73j/j mice showed considerably lower declines in stop flow pressure and superficial nephron glomerular filtration rates in response to saturating increases in tubular perfusion flow. Instead of any problems with adenosine receptor activation, the observed impairments in tubuloglomerular feedback responses were caused by lower amounts of extracellular adenosine. All things considered, it was determined that CD73 plays a significant role in facilitating communication between the macula densa and the underlying smooth muscle cells [Colgan SP, 2006]. Following renal IP, renal adenosine levels were almost 10-fold greater than at baseline. We repeated this experiment using CD73 gene-targeted mice to show that CD73 contributes to elevated tissue-adenosine synthesis with IP. Although basal adenosine levels were lower in CD73 animals than in littermate controls, elevations in extracellular adenosine with IP were markedly reduced. When considered collectively, these results show that CD73 is essential for raising renal adenosine levels during IP [Grenz A. et al., 2007]. Since an increase in cAMP is linked to improved barrier function, adenosine enhances the vascular barrier [Colgan SP, 2006].

2. Cluster of differentiation 73 (CD73) and Solid Organ Transplantation

An effective immunomodulatory molecule that builds up in inflammatory conditions is extracellular adenosine. The ectonucleotidases CD39 and CD73 work together to hydrolyze nucleotides like adenosine triphosphate and adenosine diphosphate released from damaged and necrotic cells into adenosine monophosphate and adenosine. Purinergic signaling may have a role in the inflammatory response that occurs in acute rejection and chronic allograft malfunction, according to mounting data. In a number of solid organ transplant models, changing the purinergic pathway has been demonstrated to affect graft survival and the body's reaction to ischemia-reperfusion injury (IRI). The purinergic pathway is also fundamental to the biology and operation of B and T cells. B cells have a part in chronic allograft loss, despite the fact that T cells have historically been thought to be the masterminds behind acute allograft rejection [Dwyer K.M. et al, 2014].

It is crucial to understand the function of the ectonucleotidases CD39 and CD73, as well as adenosine signaling, in solid organ transplantation, as well as how they affect IRI and T and B cell biology. Patients with advanced organ illness are given the best chance of survival by receiving a solid organ transplant. Long-term graft survival has not altered considerably [Dwyer K.M. et al., 2014] despite improvements in short-term graft survival associated with more effective immunosuppression. Evidence links recurring episodes of acute allograft rejection to the etiology of chronic allograft dysfunction and failure, despite the fact that the source of these conditions is still not fully understood. All but a few patients require lifelong immunosuppression, and there must be a careful balance between the amount of immunosuppression needed to maintain graft integrity and the undesirable side effects brought on by excessive exposure. Immunological tolerance is still the only treatment option for organ transplantation that allows for long-term allograft survival without immunosuppressive effects. In 2014, [Dwyer K.M. et al.]

There are a variety of factors that have been found to raise graft immunogenicity and rejection risk. An instantaneous, ferocious, and fulminant immune reaction known as hyperacute rejection is triggered by the recipient's immune system being exposed to the vast immunological discrepancy seen in xenotransplantation (HAR).

Research is still heavily focused on ways to reduce such events' immunological responses in recipients without increasing the need for immunosuppression. T cell activation is a specific target of the immunosuppressive treatments used today to stop allograft rejection. The outstanding 1-year graft survival of transplanted organs serves as a testament to the efficacy of this strategy. Although short-term survival has increased, long-term graft survival has remained same, and these drugs are far less effective at preventing chronic rejection. B cells have recently been recognized as having a role in chronic rejection, although they may also be helpful for the transplant. By boosting the main T cell response and encouraging regulatory T cell (T-reg) activity, B cells can modify the T cell response [Dwyer K.M. et al., 2014].

Inflammatory conditions significantly increase the pericellular levels of the innate immunomodulatory substance adenosine. The ectonucleotidase NTPDase family, of which CD39 (NTPDase1) is the prototype, hydrolyzes nucleotides including adenosine triphosphate (ATP) and adenosine diphosphate (ADP) to produce adenosine monophosphate (AMP) in wounded and necrotic cells. The 5’ ectonucleotidase CD73 then hydrolyzes AMP to adenosine. Four G protein-coupled receptors—A1, A2A, A2B, and A3—transmit adenosine's signal. In contrast to A2AR and A2BR, which are connected to the G-stimulatory subunit and cause an increase in intracellular cAMP upon activation, A1R and A3R are coupled to the G-inhibitory subunit [Dwyer K.M. et al., 2014]. Similar to CD73, which was first employed as a surface marker to distinguish distinct subsets of B cells during particular phases of development, resting B cells, T cells, including T-reg, neutrophils, NK cells, monocytes, and macrophages have also been shown to express CD73. On resting B cells, CD39 and CD73 are coexpressed; however, when B cells are activated, CD73 expression is downregulated. These cells' capacity to produce adenosine promotes immunoglobulin diversity through class switch recombination, which is crucial for establishing a humoral immune response and may affect transplant survival. T-reg co-expresses CD39 and CD73 within the T cell population. The pattern of adenosine receptor expression on both cellular groups has also been described. T cells naturally express the A2AR, and after activation, this expression is increased. The significance of the A2AR on T-reg function has just come to light. Although B cell growth is inhibited by signaling via the A3R, B cells express the A1, A2A, and A3R [Dwyer K.M. et al., 2014]. There is growing evidence that purinergic signaling contributes to persistent allograft malfunction and the inflammatory response that follows rejection. Experimentally, graft survival and the response to IRI have changed as a result of purinergic pathway modification in a number of solid organ transplant models. Additionally, the purinergic system is integral to the biology of both B and T cells, two cell subsets important for preserving allograft survival. It's crucial to consider how ectonucleotidases (NTPDase1/CD39 and CD73) and adenosine signaling affect IRI and lymphocyte biology in solid organ transplantation [Dwyer K.M. et al, 2014].

The best method of renal replacement therapy for people with end-stage renal illness is still kidney transplantation. Similar to other solid organ transplants, the development of chronic allograft malfunction, which in the kidney shows as interstitial fibrosis and tubular atrophy, lowers long-term survival rates.

Acute rejection, which is elevated in patients with delayed graft function and which in turn is impacted by IRI particularly with longer cold preservation durations, is one of the risk factors for late allograft loss [Dwyer K.M. et al, 2014].

Prior to renal ischemia, preconditioning with CD73 is crucial. Interesting protection against damage was described in CD73-deficient mice by Rajakumar et al. In this model, animals received 24 hours of reperfusion after 18 minutes of ischemia, which caused mild damage in WT mice. Nevertheless, CD73KO animals were protected against harm, and pre-treatment of WT mice with a CD73 inhibitor had a similar outcome. These findings suggest that AMP has direct biological activity at the A1R, which has just recently been attributed in vitro. In 2014, [Dwyer K.M. et al.]

Regarding adenosine signaling, parenchymal A2BR signaling enhances post ischemia blood flow limiting injury whereas A2AR activation reduces inflammation inherent in this injury.

Pretreating mice with an A2AR agonist dramatically reduces the burden placed on CD4+ T cells, which are crucial in mediating renal injury [Dwyer K.M. et al., 2014]. Renal damage was significantly reduced when A2AR agonist therapy prevented dendritic cell-mediated NKT cell activation. The transcription factor FoxP3 and the co-expression of CD4 and CD25 have historically been used to identify regulatory T cells. It has also been demonstrated that this fraction expresses CD73 and A2AR. Four T cell subsets are identified by CD4, CD25, and CD39 expression in humans and may be longitudinally monitored in peripheral blood of kidney transplant recipients [Dwyer K.M. et al., 2014].

3. Implications in Renal Ischemia

Cardiovascular morbidity and death are dramatically impacted by acute renal failure brought on by ischemia. Extracellular adenosine has been suggested to function as an anti-inflammatory metabolite, especially in situations when oxygen availability is constrained, such as ischemia. The rate-limiting enzyme for the production of extracellular adenosine is ecto-5′-nucleotidase (CD73). The ischemia preconditioning (IP) of the kidneys is aided by CD73-dependent adenosine synthesis [Grenz A. et al., 2007]. Following the first discovery that renal IP induces mouse CD73 mRNA, protein, and function, its function in IP-mediated kidney protection was investigated. In fact, CD73 mice exhibit reduced increases in renal adenosine concentration after IP. Additionally, renal protection by IP was completely restored in CD73 mice after reconstitution with soluble 5′-nucleotidase, as evidenced by clearance studies, plasma electrolytes, and renal tubular destruction. These findings indicate that pharmacological inhibition of CD73 or its targeted gene deletion abolished renal protection by IP. A. Grenz et al., 2007.

Finally, intraperitoneal administration of soluble 5′-nucleotidase to wild-type mice reduced renal damage following ischemia to a level comparable to that of IP. Together, our findings indicate that CD73 has a hitherto unknown role in protecting the kidneys from ischemia and point to the use of soluble 5′-nucleotidase as a novel therapeutic strategy for the treatment of renal disorders brought on by oxygen deprivation. The morbidity and mortality of cardiovascular patients are greatly impacted by acute renal failure, which is brought on by low oxygen availability, such as ischemia, and hypoxia. For instance, surgical operations involving the cross-clamping of the aorta and renal arteries, such as those performed during aneurysm repair or surgery for peripheral vascular disease, are linked to a 15 to 30% renal failure rate. Similar to this, 1–10% of patients experience acute renal failure following heart surgery, which is linked to greater rates of mortality and resource use. Acute renal failure can occur in up to 40% of high-risk patients who have open heart surgery, despite receiving the best possible

medical care and using renoprotective measures. Therefore, there is an urgent need for novel treatment approaches to stop renal ischemia-induced harm, and research into the molecular causes of renal ischemic injury is a hot topic. Ischemia preconditioning is a very effective experimental technique to increase kidney ischemic tolerance (IP). In actuality, preparation with brief ischemia significantly boosts the kidneys' resilience to a second ischemic insult. The underlying molecular mechanisms of renal protection by IP are mainly unknown, despite the fact that several attempts to discover them have been made [Grenz A. et al, 2007]. By releasing renal tubular TGF-1, the volatile anesthetic isoflurane guards against renal ischemia and reperfusion injury. We investigated whether renal tubular ecto-5'-nucleotidase (CD73), which is induced by TGF-1 produced by isoflurane, and adenosine can protect against renal ischemia and reperfusion injury. In mouse kidney and cultured proximal tubule cells, isoflurane enhanced adenosine synthesis and stimulated new CD73 production. Additionally, isoflurane-mediated increase of CD73 activity was inhibited by a TGF-1-neutralizing antibody [Kim M. et al, 2013].

Extracellular adenosine may have an anti-inflammatory function, particularly under conditions of low oxygen availability, according to recent studies on tissue protection. The primary method by which ecto-5′-nucleotidase produces extracellular adenosine is through the phosphohydrolysis of adenosine 5′-monophosphate (AMP) (CD73). While CD73-dependent adenosine synthesis is essential for controlling tubuloglomerular feedback, its function in renal ischemia tissue protection is still debatable. Studies that took a chimeric approach revealed that activating the A2A adenosine receptors (A2AAR) on bone marrow-derived blood cells could protect the kidneys from ischemia and reperfusion. A study that used a genetic strategy in mice with the A1AR gene targeted discovered reduced necrosis and inflammation during renal ischemia and reperfusion injury, demonstrating that extracellular adenosine provides tissue protection during renal ischemia. Contrarily, a pharmacologic investigation utilizing the 5′-nucleotidase inhibitor 5′-(,-methylene) diphosphate (APCP) demonstrated renoprotective effects by inhibiting adenosine production in rats who had undergone renal ischemia and reperfusion [Grenz A. et al, 2007]

We investigated the role of CD73-dependent adenosine production in renal protection by IP using a novel model of in situ IP that combined genetic and pharmacologic techniques in response to these contradictory findings. In this model, the intermittent blockage of the renal artery in mice is carried out using a hanging-weight method. Systematic analysis of this method reduced the variability associated with earlier methods by demonstrating highly reproducible renal damage and protection by IP. et al., 2007; Grenz, A. To show that the loss of ecto-5′-nucleotidase enzyme activity causes the decline in renal functioning measures in CD73 animals and to serve as a proof of concept. Together, these findings support our genetic research suggesting CD73 is a key factor in raising renal resistance to ischemia following IP. When soluble 5′-nucleotidase is used, ischemia-induced renal hemodynamics in CD73 mice are improved. A. Grenz et al., 2007.

In comparison to mice given anesthesia with pentobarbital, isoflurane-anesthetized mice exhibited significantly lower plasma creatinine and lessened renal tubular necrosis, neutrophil infiltration, and apoptosis. In CD73-deficient mice, mice pretreated with a selective CD73 inhibitor, or mice treated with an adenosine receptor antagonist, isoflurane was unable to prevent renal ischemia and reperfusion injury [Kim M. et al, 2013]. We then investigated nucleotidase therapy of renal ischemia in wild-type mice after demonstrating that renal protection by IP may be successfully restored in CD73 mice. Treatment with 5′-nucleotidase offered kidney protection on par with IP. Renal histology supported the protection against ischemia that we had previously seen with IP. Together, these findings propose soluble 5′-nucleotidase as a novel therapy for acute renal ischemia and show for the first time a therapeutic impact of treatment with soluble 5′-nucleotidase in renal ischemia [Grenz A. et al, 2007].

The TGF-1-neutralizing antibody or the CD73 inhibitor reduced the effectiveness of isoflurane's ability to prevent HK-2 cells from apoptosis. In order to prevent renal ischemia and reperfusion injury, isoflurane induces renal tubular CD73 and adenosine production in a TGF-1 dependent manner. According to Kim M. et al. (2013), altering

this route may have significant therapeutic ramifications for lowering morbidity and mortality associated with ischemic acute renal damage.

4. Functions in the Endocrine system:

1. ROLE of CD73 IN ENDOCRINOLOGY or ENDOCRINE DISEASES

1. Diabetic Nephropathy

The primary mechanism by which extracellular adenosine is produced is by the ecto-5'-nucleotidase (CD73), which phosphohydrolyzes nucleotides. Four different adenosine A receptors then transmit extracellular adenosine's signal (Adora1, Adora2a, Adora2b, or Adora3). Here, we proposed a functional role for extracellular adenosine's CD73-dependent synthesis and accompanying signaling during diabetic nephropathy. In the kidneys of diabetic mice, levels of CD73 protein and transcript were increased. Although soluble nucleotidase therapy was effective, genetic deletion of CD73 was linked to more severe diabetic nephropathy. Renal adenosine receptor transcript levels indicated a preferential elevation of Adora2b during diabetic nephropathy. Adora2b expression was confined to the vasculature and elevated following streptozotocin therapy in a transgenic reporter mouse. Studies in mice with tissue-specific deletion of Adora2b in tubular epithelia or vascular endothelia implicated endothelial Adora2b signaling in protection from diabetic nephropathy. Adora2b mice exhibited more severe diabetic nephropathy.

2. Type 2 diabetes mellitus

The control of the microenvironment through adenosine and with the involvement of CD39, CD73, and A2A is one of the strategies used by regulatory T cells to decrease the inflammatory response. This study examined the expression of CD73 and A2A in immune cells as well as how an adenosine analogue activated A2A and affected apoptosis in patients with obesity and type 2 diabetes (T2D). By using flow cytometry, the expression of CD73 and A2A in lymphocyte subpopulations from patients with obesity (n = 22), T2D (n = 22), and healthy individuals (n = 20) was examined. Apoptotic cells were discovered using Annexin V after lymphocytes were treated with either the selective A2A antagonist (ZM241385) or the selective A2A agonist (CGS21680). In the various investigated lymphocyte subpopulations, there is a higher expression of CD39 together with a lower expression of CD73 in the patient groups with obesity and T2D as compared to the control group. A2A expression was discovered to be elevated in various lymphocyte subpopulations from T2D patients. Positive relationships between age and BMI and CD39+ cells have also been found. The relationships between age, WHR, BMI, FPG, HbAc1, triglycerides, and cholesterol were inversely correlated with CD73+ cells. Additionally, compared to groups with obesity and controls, T2D patients had a higher percentage of apoptotic cells. Additionally, when given the A2A agonist, the CD8+ T cells of T2D patients showed less apoptosis. In conclusion, CD73 and A2A may have a role in inflammation seen in people with T2D and obesity that is mediated by apoptosis. [D. P. Portales et al., 2015]

3. Pancreatic malfunction

Cancers with high levels of the immune-suppressing molecule CD73 have lower survival rates. The prognostic significance of CD73 in pancreatic ductal adenocarcinoma is still poorly understood (PDAC). The goal of this study

was to look at CD73's predictive value in PDAC. 110 PDAC patients who had aggressive treatment made up the study's subject population. The construction and immunohistochemical staining of tissue microarray blocks with CD73 antibody. It was determined how many stained blood vessels, stroma, inflammatory cells, and tumor cells there were overall. PD-L1, perineural invasion, and histopathological grade were all positively correlated with high levels of CD73 expression in tumor cells. Lymph node metastases and CD73 positivity in tumor-infiltrating lymphocytes were substantially correlated. According to Tahkola K. et al. (2020), lymphocytic CD73 positivity was also linked to staining positivity in stromal and vascular tissues. The seventh-deadliest cancer in the world is PDAC. Due to advanced disease, over 80% of patients have an unresectable tumor when they are diagnosed, and survival statistics are still poor even after attempted curative surgery. The outcomes in PDAC have been poor [Tahkola K. et al, 2020] despite the positive results of immune-modulating medicines in many other malignancies.

Additionally, there was a correlation between stromal CD73 positive and vascular architecture. Tumor cells that were CD73 positive did not significantly correlate with CD73 positivity in any other cell types. PD-L1 expression was connected to the main tumor's histopathological grade ($p = 0.033$), T class ($p = 0.016$), and CD73 staining positivity in the stroma ($p = 0.007$). Poor disease-specific and overall survival were also substantially correlated with CD73 positivity in tumor cells ($p = 0.021$ and $p = 0.016$, respectively). In a multivariate study, low immune cell score, histopathological grade, TNM stage, and CD73 positivity in tumor cells all contributed to a poor prognosis. In summary, regardless of the quantity of tumor-infiltrating lymphocytes or TNM stage, elevated CD73 expression in tumor cells is linked to poor survival in PDAC [Tahkola K. et al., 2020]. It has been demonstrated that the tumor microenvironment affects how cancer develops. It is well known that malignant tumors like pancreatic ductal adenocarcinoma (PDAC) acquire a number of methods to inhibit the host immune system. According to earlier studies, the quantity of tumor-infiltrating lymphocytes (TILs) and survival in many cancer types are related [Tahkola K. et al, 2020].

One of the most important nucleotide metabolizing enzymes, CD73, also known as ecto-5'-nucleotidase (NT5E), is crucial for maintaining immunological homeostasis. Adenosine monophosphate (AMP) is dephosphorylated to adenosine, which activates particular G protein-coupled receptors (GPCR) and inhibits immunological response. It has been demonstrated that PDAC exhibits a shift in the distribution of CD73 from its normal apical location to a more diffuse distribution. This presumably encourages metastasis, angiogenesis, and cancer cell aggression. There are evidence to support the idea that CD73 stimulates cancer cell migration and proliferation in addition to its enzymatic activity [Tahkola K. et al., 2020]. CD73 also has non-enzymatic roles in cells. There have been reports of a link between poor survival and CD73 upregulation in a number of cancers. Contrary correlations, however, have also been noted. One explanation could be that CD73 is expressed in many different cell types, including fibroblasts, cancer cells, fractions of epithelial cells, lymphatic and blood endothelial cells, and specific populations of lymphocytes. These prognostic assessments frequently do not account for cell-specific expression. There are still limitations to CD73's predictive use in PDAC [Tahkola K. et al, 2020].

5. Functions in the Reproductive system

1. Human breast cancer progression

Extracellular 5'-nucleotidase (CD73) expression was first assessed in breast cancer tissues and cell lines, and then interfered with or overexpressed by recombinant lentivirus in cell lines in order to determine the function and investigate the mechanism of CD73 in the growth of human breast cancer. With the aid of the colony formation assay, CCK-8, and flow cytometry, the effects of CD73 on breast cancer cell proliferation and the cell cycle were examined. Adenosine, adenosine 2A receptor antagonist (SCH-58261), adenosine 2A receptor agonist (NECA), CD73 enzyme inhibitor (APCP), and Akt inhibitor were used to evaluate the interaction between CD73 and the AKT/GSK-3/-catenin pathway (MK-2206). Additionally, using a nude mouse model of human breast cancer transplantation, the impact of CD73 on breast cancer growth in vivo was investigated. The findings demonstrated

that breast cancer tissues had significant levels of CD73 expression, which increased with more advanced tumor grades and lymph node status. More malignant cells expressed CD73, and both in vivo and in vitro, CD73 overexpression increased the growth of breast cancer cells. Through CD73 enzyme activity and other mechanisms, it stimulated the AKT/GSK-3/-catenin/cyclinD1 signaling pathway. The 2017 [Zhou P. et al]

The enzyme CD73 has been linked to tumor growth in a variety of cancer forms, although studies into the prognostic value of this enzyme have yielded conflicting results. The authors showed that breast cancer cells had high amounts of CD73, and that these levels were considerably greater in tumors with advanced stages. They discovered that increasing CD73 expression encouraged cancer growth in both mouse models and cell culture. They then demonstrated how CD73 triggered the signaling pathway that promotes tumor growth, AKT/GSK3beta/beta-catenin. Consequently, the presence of CD73 may be a crucial prognostic indicator for breast cancer. The 2017 [Zhou P. et al]

The primary cause of cancer-related death in women worldwide is breast cancer. With a trend toward younger age groups, its incidence rate has been increasing. Breast cancer is one tumor type that overexpresses ecto-5'-nucleotidase (CD73), a 70 kDa glycosylated protein attached to the outer surface of the plasma membrane by a glycosyl phosphatidyl inositol (GPI) anchor. 2-5 The primary job of CD73 is to catalyze the conversion of extracellular 5'-AMP to adenosine, which is crucial in a variety of physiological and pathological situations (A1, A2A, A2B and A3 AR). 6 Up-regulation of CD73 has been linked to tumor-promoting properties, treatment resistance, and cancer phenotypes that are highly invasive. 7 We discovered that CD73 might, depending on the activity of the enzyme, either encourage or inhibit the growth and metastasis of human breast cancer cells. Two essential signal pathways for cell growth and development, PI3K/Akt and Wnt/-catenin, are also key players in cancer. 8-10 The two pathways "crosstalk" with one another [Zhou P. et al., 2017]. As between 10 and 20 percent of all breast tumors, triple-negative breast cancer (TNBC) is distinguished by the absence of HER2/neu expression and hormone receptors (estrogen and progesterone receptors). TNBC are typically linked to poor clinical outcomes and are not eligible for hormonal therapy or Herceptin/trastuzumab targeted therapy. The main systemic therapy for TNBC patients is anthracycline/taxane-based neoadjuvant chemotherapy, but resistance to this therapy is widespread, therefore finding novel potential therapeutic agents is necessary to enhance their prognosis [Lafont V, 2018].

According to a recent assessment of the literature, there is ongoing debate regarding the prognostic value of CD73 expression in various human solid tumors. According to two meta-analyses involving 13 and 14 studies and 12,533 and 2,951 individuals with solid tumors, respectively, it is mostly linked to inverse overall survival (OS) and disease-free survival when CD73 is highly expressed (DFS). CD73 has also been linked to poor prognosis in research on prostate, colorectal, gastric, gallbladder, and other malignancies. Quite the opposite, CD73-high expression indicated better prognosis, lower stage, and higher degree of differentiation for epithelial ovarian carcinoma, stomach and bladder malignancies, and rectal adenocarcinoma. Due to the significant variability of breast tumors, it is currently unclear whether CD73 is associated with long-term survival in this disease. In 136 stage I-III breast cancer patients, Supernat et al. found that positive CD73 expression is related to longer DFS and OS. Contrarily, it has been demonstrated that CD73 expression is substantially related with a poor prognosis, particularly in TNBC, utilizing gene-expression analysis of over 6,000 breast cancer cases. The results of this study also showed a link between higher doxorubicin resistance in TNBC patients and CD73 expression. It's interesting to note that anti-CD73 antibody therapy improved the anti-tumor immune response caused by doxorubicin, indicating CD73 as a viable novel target in TNBC. A2a receptor antagonists and monoclonal anti-CD73 antibodies are currently undergoing clinical trials, therefore it's critical to better pinpoint the individuals who will benefit from these novel immune-based treatments [Lafont V, 2018].

Buisseret et al. recently published the findings of a multiparametric analysis they conducted on a sizable group of TNBC patients. In 122 TNBC samples from the Breast International Group (BIG) 02-98 adjuvant prospective phase III clinical study comparing the addition of docetaxel to doxorubicin with doxorubicin-based treatment in

node-positive breast tumors, they quantitatively evaluated CD73 expression. In this work, the scientists examined the expression of CD73 on tumor cells, stromal cells, or immune cells and assessed its predictive value based on the expression of CD45, cytokeratin, and CD73 by multiplex immunofluorescence. Despite the fact that CD73 was expressed in all compartments, they found that tumor and immune cells had higher levels of CD73 expression than stromal cells did. Additionally, tumor and immune cells expressed CD73 at higher levels in patients who had extensive lymph node invasion. Poorer survival was associated with increased CD73 expression on tumor cells, but there was no discernible link between stromal or immunological CD73 expression and clinical outcome. A CD45+ area in relation to the tumor tissue area was determined using an algorithm based on cytokeratin and CD45 positivity while the scientists also studied tumor immune infiltration. According to a prior study, greater immune infiltration was linked to improved DFS and OS. The study of both CD73 expression and CD45+ area demonstrated a negative connection between the level of immune infiltration and CD73 expression on tumor and immune cells, but not on stromal cells. The previously hypothesized predictive utility of CD73 gene expression for resistance to anthracycline-based treatment, however, was not supported by this investigation [Lafont V, 2018].

The authors were able to isolate patient subgroups with various prognoses using the concurrent analysis of CD73 expression on tumor cells and tumor immune infiltration level. Four phenotypic patient groupings were identified from this investigation, but only three subgroups in terms of clinical outcome. As a result, patients with a good prognosis (strong immune infiltration and low CD73 expression) and those with a bad prognosis were separated in this retrospective analysis (low immune infiltration and high CD73 expression). Results from the combined examination of the two factors may be useful for developing novel TNBC treatment strategies. A therapy targeting CD73, either alone or in combination with immune checkpoint inhibitors like anti-PD1, anti-PDL-1, or anti-CTLA-4 antibodies, should be beneficial for patients who have both a high immune infiltrate and a high expression of CD73. This will remove the immunosuppressive brake and strengthen an already-existing immune response. A combination of CD73-targeting therapy with medications that can boost tumor infiltration as a vaccination or through adoptive cell transfer may be a superior therapeutic choice for patients with poor immune infiltration [Lafont V, 2018]. Additionally, CD73 plays a species-specific role in arterial calcifications in humans, which is not replicated in CD73-deficient mice. In endometrial cancer, CD73 downregulation in human malignancies has been reported. Loss of it is linked to more virulent illness and worse overall survival. Adenosine produced by CD73 in a normal endometrium protects the integrity of the epithelium, and when CD73 is lost, the lack of cell-cell adhesions facilitates the growth of tumors. In contrast, myoepithelial cells rather than differentiated cells express CD73 in normal breast tissue. Because they resemble stem cells and behave aggressively like tumor cells, myoepithelial cells. This is supported by the fact that CD73 is elevated in triple-negative breast cancer (TNBC) cancer cells, which exhibit a gene expression pattern like that of basal/myoepithelial cells. 2020 Harvey J.B. Here, we demonstrate that triple-negative breast tumors with high CD73 expression have a worse prognosis based on gene-expression data from over 6,000 breast cancer patients (TNBC). We looked into how CD73 and anthracycline efficacy relate as anthracycline-based chemotherapy regimens are the standard of care for TNBC. High CD73 gene expression was substantially related with a poorer rate of histological complete response or the elimination of invasive tumor at surgery in TNBC patients treated with anthracycline-only preoperative chemotherapy. We showed that CD73 overexpression provided chemoresistance to doxorubicin, a common anthracycline, by dampening adaptive antitumor immune responses via activation of A2A adenosine receptors in breast cancer mice models. Mice with established metastatic breast cancer had considerably longer survival times thanks to targeted CD73 inhibition, which also improved doxorubicin-mediated antitumor immune responses. When considered collectively, data indicate that CD73 is a therapeutic target for TNBC [Loi S. et al, 2013].

Patients with solid malignancies, including TNBC, are currently being tested for the efficacy of a number of inhibitors that target CD73 or the adenosine A2a receptor. Initial research with adenosine A2a receptor antagonists revealed acceptable tolerability and an increase in immune cells that infiltrate tumors. Three clinical trials using monoclonal antibodies that disrupt CD73 are currently being conducted in relation to medications

that target CD73. A variety of tumor microenvironmental subpopulations underwent significant changes, including an increase in CD8 effector cells and activated macrophages [Lafont V, 2018]. Overall, the findings from Buisseret et al. show the utility of tracking CD73 expression on various cell subtypes and combining it with other criteria like immune infiltration. They also support its value as a biomarker in TNBC. This research adds to the evidence that CD73 expression in human TNBC is linked to a poor prognosis and diminished anti-tumor immunity, and that targeting CD73 may be a potential way to rewire the tumor microenvironment in TNBC [Lafont V, 2018]. Contrarily, there are numerous publications that link CD73 to a better clinical outcome in patients with various solid malignancies. Increased CD73 expression may be used as a potential diagnostic marker for breast cancer with a favourable prognosis, according to a study examining the biological relevance of CD73 in breast cancer. Another study looking at CD73 expression in epithelial ovarian cancer revealed higher levels of CD73 expression in patients with a good prognosis. This study [Ranjbar M-A. et al, 2019] showed associations between CD73 overexpression and better prognosis, lower stage, and better differentiation.

15% to 20% of all breast tumors are classified as triple-negative breast cancer (TNBC), which is indicated by the lack of the HER2 gene, the progesterone receptor (PgR), and the estrogen receptor (ER) gene expression. TNBC differs from other breast cancer subtypes in that it has a worse prognosis and a higher chance of spreading to important organs. Given that the progress of poly (ADP ribose) polymerase and EGFR inhibitors has been unsatisfactory thus far, it appears that there are few therapeutic alternatives on the horizon and no identified biological targets for this class of breast cancer patients. Because of this, treating TNBC is a big issue in modern clinical practice, and finding therapeutic targets is a critical area of clinical need. 2013 [Loi S. et al]

Anthracyclines need tumor cells to release ATP into the extracellular space, which is a process triggered by autophagy, in order to stimulate antitumor immune responses. CD39 (ENTPD1) catalyzes the hydrolysis of ATP to ADP, and CD73 (NT5E) catalyzes the hydrolysis of AMP to adenosine, a strong immunosuppressant. Although NDP kinase and adenylate kinase adversely limit CD39 activity, CD73 activity is irreversible, placing CD73 at a critical checkpoint in the conversion of ATP to adenosine. Through the activation of high affinity A2A adenosine receptors, CD73 further inhibits immunological responses by raising extracellular levels of adenosine [Loi S.et al, 2013]

It has been proven that CD73 expression in breast cancer patients has clinical significance. We discovered that high CD73 expression is substantially related with a poor prognosis, particularly in TNBC, using gene-expression analysis on over 6,000 breast cancer cases. Additionally, our data showed that CD73 expression in TNBC patients is linked to an increased resistance to the anthracycline chemotherapy drug doxorubicin (DOX). We show that DOX treatment increases the expression of CD73 on tumor cells using animal models and human TNBC cell lines. CD73 suppresses IFN-producing CD8+ T cells by activating A2A adenosine receptors, hence promoting DOX resistance. Thus, our research demonstrates a distinct mechanism of chemoresistance caused by the buildup of extracellular adenosine and identifies CD73 as a novel therapeutic target for TNBC. Therefore, cytotoxic chemotherapy, particularly anthracycline-based regimens, continues to be the cornerstone of TNBC treatment today. Despite having a dismal prognosis, some TNBC patients appear to benefit from anthracycline-based treatment, which indicates a high level of genomic heterogeneity within this cohort. Unfortunately, little is yet known about the molecular processes that underlie this heterogeneity and how it affects how well a patient respond to treatment. A recent study on the mechanism of action of anthracyclines may give new information on potential chemoresistance mechanisms. Increasing evidence indicates that anthracyclines mediate their anticancer effectiveness not only through direct cytotoxic effects but also by activating adaptive antitumor immune responses by generating an immunogenic kind of tumor cell death. Anthracycline treatment in mice requires IFN-producing CD8+ T cells for effective action. High intratumoral numbers of IFN- and CD8+ T cells are linked to superior clinical responses to anthracycline-based chemotherapy regimens, according to correlating clinical studies [Loi S. et al, 2013], which further support this finding.

Although CD73 expression in numerous cancers, including breast cancer, has been confirmed, there has only been a limited amount of research linking it to clinical outcome. Here, we demonstrate that the TNBC subtype of breast cancer exhibits increased CD73 expression, and that TNBC patients with high CD73 expression have a higher risk of developing distant metastases, the primary cause of breast cancer death. Although there have been some attempts to switch away from anthracycline-based regimens, anthracycline-based chemotherapy has been and still is the gold standard of care for TNBC. In some studies, CD73 was linked to anthracycline treatment resistance. Anthracyclines are a subclass of chemotherapeutic drugs that are capable of enhancing antitumor immune responses, hence CD73-mediated immunosuppression may be able to inhibit DOX efficacy in immunocompetent animals. In vivo, DOX's therapeutic efficacy is inhibited by CD73-mediated immunosuppression [Loi S. et al., 2013]. The observations involving CD73 expression in prostate, stomach, and some forms of breast cancer are supported by additional investigations, though. The fact that different tissues have varying levels of CD73 enzymatic activity may help to explain inconsistent data regarding the function of CD73 in diverse solid tumors [Ranjbar M-A. et al., 2019].

2. Ovarian cancer and other solid tumors

A new study demonstrating the expression and potential immunosuppressive effect of CD73 in human ovarian cancer specimens' ex vivo and cell lines in vitro further supports the therapeutic significance of CD73 in ovarian cancer. Adenosine production by ovarian cancer cells was strikingly higher than that of resting or activated T-regs. Importantly, in a mouse xenograft model of human epithelial ovarian cancer, blockage of CD73 with the selective inhibitor APCP (, -methylene adenosine 5'-diphosphate) enhanced the in vivo antitumor effects of T cells and improved overall survival. Additionally, melanoma tumors have significant levels of CD73 expression [Zhang B., 2012]. Although several host cell types, including subsets of lymphocytes, endothelial cells (EC), and dendritic cells (DC), express CD73, it has not yet been fully understood how host CD73 expression and activity contribute to the development of cancer. Below is a summary of these three autonomous groupings, which are strikingly similar and complimentary to one another [Zhang B., 2012]. It appears that CD8+ T cells are necessary for the protective impact of host CD73 loss on initial tumors, and that this effect is linked to elevated endogenous antitumor T cell immunity. In animals lacking CD73 expression, CD8+ T cell infiltration into tumor tissue may be increased [Zhang B., 2012].

The higher degree of differentiation, lower stage, and better prognosis for epithelial ovarian carcinomas were all predicted by CD73-high expression. Nonmuscle invasive bladder cancer has also been documented to have a similar outcome. However, additional studies in triple negative breast cancer, colorectal, gastric, gallbladder, and prostate cancer demonstrated that CD73 was an adverse predictive factor. A systematic review and meta-analysis of previously published research were undertaken in order to more precisely assess the prognostic value of the CD73-adenosinergic pathway in solid tumors [Wang R. et al., 2017]. Hausler et al. recently revealed that anti-CD73 mAb improved polyclonal NK cells' lytic activity against human ovarian cancer cell lines. Similar to this, CD4+ T cell proliferation was considerably boosted when CD73 by mAb was blocked in coculture with ovarian cancer cells. As a result, anti-CD73 therapy enhances the immune system's ability to kill ovarian cancer cells. Anti-CD73 treatment appears to be mostly dependent on its ability to stimulate in vivo antitumor immune responses, according to Zhang H-Z et al. (2014).

Serious ovarian cancer patients with increased CD73 expression and BRAF mutation have improved clinical outcomes [Harvey J.B., 2020].

6. Functions in the Respiratory system:

1. ROLE of CD73 IN RESPIRATORY DISEASES

The respiratory system is the one where CD73's role in maintaining barrier function is most obvious because there, it reduces endothelial permeability in an adenosine-dependent manner. During heart failure and myocardial infarction, CD73 plays a critical role in cardioprotection. Rapid and sustained upregulation of CD73 affords protection in the liver and kidney under ischemia-reperfusion damage circumstances [Minor M. et al., 2019].

In other instances, it is unclear how CD73 mediates tissue harm. For instance, CD73 promotes liver fibrosis while acting as a protective factor in lung fibrosis. Future research that combines physiological responses with CD73 regulation and function at the cellular level will increase its usefulness as a disease target [Minor M. et al, 2019].

1. Ecto-5'-nucleotidase (CD73) and HUMAN AIRWAYS

Extracellular adenosine controls epithelial activities in human airways that facilitate mucociliary clearance, a crucial airway defense mechanism against bacterial infection. Determining the mechanisms of adenosine synthesis is essential for understanding the function of this nucleoside in maintaining airway homeostasis. In this work, the origin of the adenosine found on the mucosal surface of human airway epithelia was determined. The synthesis of cytosolic and cell surface adenosine, as well as transepithelial transport, were measured in polarized primary cultures of human nasal or bronchial epithelial cells [Boucher C. et al, 2003]. Human bronchial epithelial cells did not contain messenger RNA for the cytosolic AMP-specific 5'-nucleotidase (CN-I), which suggests that mucosal adenosine did not come from intracellular pools. Ecto 5'-nucleotidase (ecto 5'-NT, CD73) and alkaline phosphatase are the two ectonucleotidases that mediate the conversion of AMP to adenosine (AP). With ecto 5'-NT and NS AP mRNA predominating in higher and lower airways, respectively, the two ectoenzymes showed opposing airway distributions. Adenosine concentrations on airway surfaces are regulated by extracellular nucleotide catalysis, ecto 5'-NT, and NS AP, according to the results of these tests [Boucher C. et al., 2003].

An important part of the airway defense against the emergence of infectious lung illnesses is mucociliary clearance (MCC). Extracellular nucleotides control a number of epithelial processes related to MCC. Due to the dearth of knowledge regarding endogenous sources of extracellular adenosine on the mucosal surface of airway epithelia, the significance of adenosine receptor-mediated control of MCC is yet unknown. Adenosine may come from the interstitial compartment and pass through the tight connections of the airway epithelium to reach the lumen. The cytosolic AMP-specific 5'-nucleotidase (CN-I) might also produce the nucleoside intracellularly, and the nucleoside could then travel to the mucosal surface via nucleoside transporters. As an alternative, many mammalian cells have been shown to release ATP and convert it to adenosine on the cell surface. Under normal circumstances and in response to mechanical stimuli such membrane stretch, shear stress, or swelling brought on by hypotonicity, human airway epithelial cells produce ATP. Under baseline conditions, the airway surface fluids contained all adenine nucleotides and nucleosides. A balance between ATP release and cell surface metabolism maintains the amounts of adenine nucleotides and nucleosides, according to research by Lazarowski et al. (Boucher C. et al., 2003). Human nasal and bronchial epithelial cells dephosphorylate exogenous ATP into ADP, AMP, and adenosine. Ecto 5'-nucleotidase (ecto 5'-NT, CD73) and alkaline phosphatases have both been implicated in the dephosphorylation of AMP into adenosine on mammalian cells. APs will metabolize a variety of substrates, such as 5'-nucleotides (ATP ADP AMP adenosine), pyrophosphate, and p-nitrophenyl phosphate, whereas ecto 5'-NT selectively dephosphorylates nucleoside monophosphates (AMP adenosine). Four ectoenzymes make up the AP family: the intestinal AP (I AP), tissue nonspecific AP (NS AP), placental AP (PLA AP), and germ-cell AP (G AP), which has been linked to malignant tumors and the testis. Since many years ago, idiopathic pulmonary fibrosis and chronic conditions like acute lung injury have been diagnosed using bronchoalveolar fluid AP activity. Furthermore, ecto 5'-NT activity in human airways has not been studied. As a result, it is yet unknown if targeting these ectoenzymes may be used to pharmacologically alter endogenous adenosine levels [Boucher C. et al, 2003].

Human airway epithelia's mucosal surface has sources of extracellular adenosine that have been studied, with a focus on penetration from the interstitial space, synthesis and secretion from the epithelial cells, and/or cell

surface metabolism of released nucleotides. On primary epithelial cultures, freshly removed airway epithelia, or bronchial sections, all tests were conducted. High-performance liquid chromatography was used to evaluate the permeability of the serosa to the mucosa and identify the species that were transferred (HPLC). Cell surface conversion of ATP, ADP, AMP, and adenosine produced adenosine. A prototypical inflammatory cytokine, interleukin-1 (IL-1), and its effects on the production of ecto 5′-NT and APs should be taken into account because adenosine may be crucial in disorders linked to airway inflammation [Boucher C. et al, 2003]. A concentrative Na+-dependent nucleoside transporter actively cleared extracellular adenosine from the mucosal surface of human nasal epithelial cells. These characteristics are in line with the vectorial transport pathway for adenosine across intestinal epithelia that has been characterized. Together, these results show that the endogenous adenosine concentrations found on the mucosal surface of human airway epithelia are not caused by interstitial or cytosolic pools of nucleosides [Boucher C. et al, 2003].

Adenosine may be primarily obtained from released ATP through cell surface metabolism, according to a wealth of literature detailing basal and induced ATP release from the mucosal surface of airway epithelia. In fact, extracellular ATP has been observed to be converted to adenosine at the cell surface in the majority of mammalian tissues. In the current study, we have demonstrated that ATP is successively dephosphorylated on the surfaces of airway epithelial cells into ADP, AMP, and adenosine. The significant inhibitory effect of 0.3 mM,-met-ADP on the baseline cystic fibrosis transmembrane regulator activity of Calu-3 cells (>70 percent) highlighted the physiological importance of constitutive ATP release and conversion to adenosine. According to one description, this substance inhibits ecto 5′-NT and AP competitively, inhibiting the conversion of AMP to adenosine on the cell surface. It was concluded that mucosal and serosal surfaces constitute different compartments with respect to nucleotide pools and purine-mediated signaling pathways since ATP, ADP, AMP, and adenosine were restricted to the epithelial surface on which ATP was delivered. A2B receptors, G proteins, the cystic fibrosis transmembrane regulator, and the location of ATP release are all located nearby, according to patch clamp tests done on Calu-3 cells. Together, our results suggest that the primary source of adenosine for P1 receptor activation on the mucosal surface of human airway epithelia is local ATP release and metabolism [Boucher C. et al., 2003].

Different cation and pH sensitivities have been observed for mammalian ecto 5′-NT and AP. Ecto 5′-NT that had been purified from rat glioma was not responsive to Ca2+ or Mg2+. Mg2+, on the other hand, increased the Mg2+-sensitive AMPase activity we found on the mucosal surface of human bronchial epithelial cells by 2-3 fold in the liver and kidney of human beings. This suggests that an AP may be in charge of the Mg2+-sensitive AMPase activity. While the alkaline activity isolated to the mucosal surface related to APs, ecto 5′-NT was responsible for the activity peak seen on both surfaces around pH 7.5 [Boucher C. et al, 2003]. Human nasal and bronchial epithelial cells from polarized primary cultures displayed the in vivo morphologic traits of proximal airway epithelia, with columnar ciliated and secretory cells overlying basal-like cells. Ecto 5′-NT would be expressed on the mucosal and serosal surfaces of airway epithelia, whereas NS AP would only be present on the mucosal surface based on the biochemical characteristics of AMP hydrolysis on these cells. Ecto 5′-NT showed polarity in rat nasal respiratory epithelium that was in line with these findings. By using histochemistry, ecto 5′-NT was specifically localized to the basal cells that line the mucosa and the mucosal surface of columnar epithelial cells. Predominantly on the apical plasma membrane of columnar epithelial cells, human airway NS AP was found. [C. Boucher et al., 2003]

Quantitative investigation of mRNA levels in human airways with cultured and recently excised epithelial preparations has revealed novel information on the tissue distribution of the AMP-hydrolyzing ectoenzymes. In bronchial epithelial cells, ecto 5′-NT and NS AP expression were 2-3 times greater than in nasal epithelial cultures. Second, we showed that the two ectoenzymes exhibit opposing expression patterns across airways using freshly removed epithelial cells. From nasal to bronchiolar epithelia, ecto 5′-NT mRNA levels steadily decreased, whereas NS AP expression rose with airway development.

The concentration of the substrate had a significant impact on the relative contribution of the two ectoenzymes that hydrolyze AMP on the mucosal surface of human bronchial epithelial cells. [C. Boucher et al., 2003] Ecto 5'-NT and NS AP both appear to have a role in the metabolism of nucleotide concentrations that are localized after ATP release from human airway epithelial cells based on their high affinity activities. Therefore, the generation of P1 receptor agonists from ATP release on airway surfaces would be rate-limited by the conversion of AMP into adenosine. [C. Boucher et al., 2003]

The interaction between nucleotide and nucleoside concentrations, ectonucleotidases, P2 and P1 receptors, and MCC on the human airway epithelial surfaces is complex. According to Boucher C. et al. (2003), mechanical stimulation, such as coughing-induced shear stress, causes local cell surface ATP concentrations to rise to levels that trigger P2 receptor-mediated MCC activities.

The synthesis of adenosine on the mucosal surface of human airway epithelial cells is mediated by ecto 5'-NT and NS AP. Ecto 5'-NT's comparatively high efficiency makes it likely that this enzyme will be crucial in controlling adenosine-mediated epithelium activities. However, all APs also dephosphorylate ADP and ATP in addition to AMP. According to Boucher C. et al. (2003), under pathological circumstances, trauma produces a significant amount of extracellular nucleotides that could harm the epithelium. For instance, it has been discovered that airway epithelial cells carry P2X7 receptors, an ATP-gated channel known to trigger apoptosis. These airways may be shielded from the harmful effects of high ATP concentrations by the high-capacity NS AP. The 5-fold increase in NS AP activity and expression caused by IL-1 suggests that this enzyme plays a part in airway defenses during times of inflammation. A possible role for NS AP in additional airway processes, such as bacterial endotoxin neutralization and sphingosine 1-phosphate receptor signaling, is suggested by the broad substrate specificity of APs [Boucher C. et al, 2003].

During the chronic phase of the disease, adenosine is quickly converted to inosine by adenosine deaminase (ADA), decreasing the impact of CD73. A useful biomarker of tuberculosis, ADA has a high quantity in the serum of people with pleural tuberculosis. Alkaline phosphatase (AP), which hydrolyzes AMP to adenosine, might still have a compensating function in CD73 KO mice. CD73 and nonspecific AP mRNAs are primarily found in the upper and lower airways of human lungs, respectively. Interestingly, tuberculosis patients had higher AP activity [Petit-Jentreau L. et al., 2015].

The participation of adenosine (ADO) in lung damage depends on the activation of its receptors. It is best to think of the ADO A2A and A2B receptors as having both tissue-protective and tissue-destructive processes. There hasn't been a successful method for identifying the mechanism(s) by which ADO changes from having tissue-protective to tissue-destructive qualities in chronic airway damage. We created a long-term CSE exposure paradigm by continuously exposing Nuli-1 cells to a 5 percent extract of cigarette smoke (CS) for three years (LTC). Significant structural modifications, reduced migration, and proliferation lead to poor airway wound closure in LTC. Further research revealed that long-term CSE exposure enhances the expression of CD73 and ADORA2B, boosts the production of ADO, and decreases the activity of PKC alpha and the p-ERK signaling pathway. ADORA2B and/or CD73 inhibition in LTC activates PKC alpha and boosts p-ERK signaling. Compared to either alone, knocking both down demonstrated a superior improvement in wound repair. [Zian, Tian, et al., 2021]

Through activating PKC alpha, lowering the number of inflammatory cells in bronchoalveolar lavage fluid and the production of the inflammatory mediator IL-6, inhibiting fibrosis-like lesions, and reducing collagen deposition surrounding bronchioles, double knockout CD73 and ADORA2B significantly improved CS-induced lung injury, according to in vivo experiments. Long-term CSE treatment increases CD73 expression and ADO production overall, which encourages low affinity ADORA2B activation and the resulting decline in PKC alpha activity and the ERK signaling pathway, as well as the suppression of airway wound healing. Additionally, research suggests that

ADORA2B and CD73 may be more effective therapeutic targets for treating chronic CS lung illnesses and poor wound healing [Zian, Tian, et al., 2021].

2. Ecto-5'-nucleotidase (CD73) and hypoxia

Only a few investigations have shown that CD73 inhibition or genetic deficiencies significantly increase hypoxia-induced vascular leak in many organs (lung, heart, gut, and kidneys) [Colgan SP, 2006]. In a number of cardiovascular-related illnesses, such as obstructive sleep apnoea, heart failure, and diabetes, increased sensory neuronal activity from the carotid body (CB) has emerged as a primary cause of hypertension. Thus, improving outcomes in these important patient groups may be achieved through the development of new targets and pharmaceutical treatment approaches intended to decrease CB sensory activity. The goal of the current investigation was to determine if the adenosine-producing enzyme ecto-5'-nucleotidase (CD73) is functionally significant in modulating CB sensory activity and cardiovascular respiratory responses to hypoxia. AOPCP, which inhibits CD73, decreased sensory activity across graded hypoxia and decreased basal discharge frequency by 76 5% in the entire CB preparation in vitro. Additionally, AOPCP dramatically reduced increases in sensory activity brought on by mitochondrial inhibition. By inhibiting adenosine receptors with 8-(p-sulfophenyl) theophylline, these effects were induced. The hypoxic ventilatory response was dramatically reduced in vivo by the administration of AOPCP. Additionally, AOPCP altered the cardiovascular reactions to hypoxia, as shown by diminished increases in heart rate and accentuated alterations in femoral vascular conductance and mean arterial blood pressure. As a result, CD73 is recognized as a new regulator of CB sensory activation. Future research is necessary to determine whether CD73 inhibition can successfully diminish CB activity in CB-mediated cardiovascular disease. (2017) [Holmes A.P. et al.]

Chronic overactivation of the carotid body (CB) has been identified as a key contributor to hypertension in a number of cardiovascular disorders, including heart failure, sleep apnea, and diabetes (Ribeiro et al. 2013; Schultz et al. 2015; Del Rio et al. 2016; Prabhakar, 2016). Therefore, the creation of cutting-edge treatment plans that surgically or pharmaceutically target the CB may enhance outcomes in these sizable patient populations. In fact, preliminary research indicates that bilateral CB excision improves exercise tolerance and lowers sympathetic outflow in people with heart failure (Niewinski et al. 2017). Unilateral CB excision in a small group of patients with drug-resistant hypertension resulted in a drop in ambulatory blood pressure in about 50% of the patients (Narkiewicz et al. 2016). The variable effectiveness of unilateral resection (Narkiewicz et al. 2016) and potential safety issues with bilateral resection, as shown by a significant drop in O2 saturation during sleep (Niewinski et al. 2017), suggest that pharmacological dampening of chemoreceptor activity may still be a more effective alternative to surgery. (2017) [Holmes A.P. et al.]

The neuronal signal that encourages ventilatory and cardiovascular reflex reactions arising from the CB, most notably in response to systemic hypoxia, is the Carotid Sinus Nerve (CSN) discharge frequency, conveyed into the CNS (Kumar & Prabhakar, 2012). Several significant neurotransmitters and neuromodulators, such as ATP, ACh, dopamine, serotonin, adrenaline, and adenosine, regulate the modification of CSN outflow (Thompson et al. 2016). In both people and animals (Conde et al. 2006; Xu et al. 2006), adenosine is a well-known CB chemostimulant (Tubek et al. 2016). In order to find new potential targets that lower CB chemoafferent activity, it may be helpful to better characterize the physiological relevance of adenosine and its production and/or signaling pathways [Holmes A.P. et al, 2017].

Following ATP's extracellular breakdown, synaptic adenosine, a neurotransmitter tonic released from the CB type 1 cell, may be produced (Piskuric & Nurse, 2013). Ecto-5'-nucleotidase (CD73) and ectonucleoside triphosphate diphosphohydrolyase 1 (CD39) are required for the conversion of ATP to adenosine (Bianchi & Spychala, 2003). In extracts of the whole rat CB, the expression of these two enzymes has been verified (Salman et al. 2016). Adenosine induces chemostimulation either by directly activating postsynaptic sensory fibers (through stimulation of the A2A receptor) (Conde et al. 2006; Xu et al. 2006; Livermore & Nurse, 2013) or by enhancing

type 1 cell excitability (by A2A and/or A2B receptors) (Nunes et al. 2014; Holmes et al. 2015). Adenosine may also be produced in type 1 cells and subsequently released into the synapse via the bidirectional equilibrative nucleotide transporter, according to another theory (ENT) (2017) [Holmes A.P. et al.]

The goal of the current investigation was to determine whether pharmacologically inhibiting CD73 or ENT successfully lowers the frequency of CSN discharges in normoxic or hypoxic environments. Furthermore, we investigated whether in vivo CD73 targeting altered the responses to hypoxia in breathing, heart rate, blood pressure, and vascular conductance. According to the research, CD73 but not ENT antagonisty reduces basal CSN frequency and dampens the response to hypoxia. Additionally, the cardiovascular and ventilatory responses to hypoxia are reduced by CD73 inhibition. As a result, we suggest that CD73e is a brand-new modulator of CB chemoafferent outflow. (2017) [Holmes A.P. et al.]

In people and animal models of sleep disordered breathing, heart failure, and diabetes, there is growing evidence that a persistent increase in CB sensory output in normoxia and hypoxia contributes considerably to hypertension and cardiac arrhythmia (Ribeiro et al. 2013; Schultz et al. 2015; Del Rio et al. 2016; Prabhakar, 2016). This is a result of the CB causing a sustained increase in sympathetic neuronal output to the heart and vasculature, which causes pro-arrhythmic cardiac autonomic imbalance and persistent vasoconstriction (Narkiewicz et al. 1998; Peng et al. 2003; Del Rio et al. 2016; Linz et al. 2016). Recent small-scale clinical investigations demonstrate that surgical unilateral or bilateral CB ablation may help some patient populations by lowering sympathetic output and arterial blood pressure (Narkiewicz et al. 2016; Niewinski et al. 2017). However, because to potential safety issues of total loss of CB sensory activity, such as excessive O2 desaturation during sleep, its use as a first-line treatment for CB-mediated hypertension may be restricted (Niewinski et al. 2017). Patients with obstructive sleep apnoea who depend on CB sensory activity to trigger arousal and restore airway patency may find this safety risk to be especially pertinent. Additionally, only 50% of hypertension individuals benefit from unilateral CB resection, which appears to only reduce blood pressure for a limited period of time (up to 12 months) (Narkiewicz et al. 2016). A safer and more effective treatment for this type of CB-mediated hypertension may therefore be found by discovering new potential pharmacological targets in the CB that can be targeted to decrease (but not abolish) chemosensory activity [Holmes A.P. et al, 2017].

CD73 mediates the frequency of CB sensory discharge under hypoxia. After CD39 first breaks down ATP and ADP, CD73 catalyzes the creation of adenosine from AMP (Bianchi & Spychala, 2003). A considerable amount of synaptic ATP is present in the CB as a result of tonic vesicular neurosecretion from type 1 cells and ATP release from type 2 cells via pannexin-1 channels (Murali & Nurse, 2016). Therefore, a decrease in adenosine synthesis is most likely what causes the attenuation of sensory discharge brought on by CD73 inhibition. In fact, we demonstrate that inhibition of CD73 was equivalent to antagonistic adenosine receptors in reducing normoxic and hypoxic CB sensory activity. The threshold needed for CB activation under hypoxia is also lowered by antagonistic effects on CD73 and adenosine receptors, which is consistent with a corresponding decline in CB hypoxic sensitivity [Holmes A.P. et al, 2017].

By activating A2A and A2B receptors on type 1 cells (Conde et al. 2006, 2008; Xu et al. 2006; Livermore & Nurse, 2013), or by stimulating the nerve ending upon binding to postsynaptic A2A receptors, adenosine produced from CD73 has the ability to modify CB hypoxic sensitivity (Conde et al. 2006). Given that CD73 will enhance extracellular adenosine in the type 1 cell-chemoafferent synapse and so have access to both pre- and postsynaptic receptors, both of these mechanisms are likely. Despite the possibility that 8SPT targets A1 receptors as well, a functional involvement in our findings is unlikely given the lack of A1 mRNA expression in the rat CB. Gauda (2000) and Kobayashi et al. (2017) [Holmes A.P. et al.]

Importantly, we also show that pharmaceutical inhibition of CD73 decreases but does not entirely eradicate chemoafferent activity in hypoxia. So, although greatly diminished, transmission of a clear hypoxic neuronal signal into the CNS would still be possible with efficient targeting of CD73. Significant CD73 mRNA expression in

the rat CB has been demonstrated and verified (Salman et al. 2016). The specific location of CD73 and CD39 has not yet been established. We propose that CD73 in the CB is likely situated similarly given the known co-localization between CD73 and A2A receptors in striatal neurones in the CNS (Augusto et al. 2013). (i.e. on type 1 cells and nerve endings) (2017) [Holmes A.P. et al.]

Contrarily, inhibition of the ENT had no impact on the frequency of either normoxic or hypoxic discharge, indicating that insufficient adenosine is produced through the ENT in normoxia and hypoxia to have a direct impact on hypoxic neuronal discharge. This is in line with past findings showing that ENT activity was not required for extracellular adenosine recovery in normoxia (Conde & Monteiro, 2004; Conde et al. 2012) (2017) [Holmes A.P. et al.]

It is yet unknown whether chronic or chronic intermittent hypoxia directly increases CD73 activity in the CB. But new research indicates that ATP and 5HT can both increase ATP release from type 2 cells, magnifying the synaptic ATP concentration (Murali & Nurse, 2016; Murali et al. 2017). Our research indicates that increased CD73 activity brought on by this elevated ATP availability would function to increase CB O2 sensitivity, which may be a factor in the exaggerated chemoafferent responses seen in chronic or intermittent hypoxia. (2017) [Holmes A.P. et al.]

In response to mitochondrial inhibition, the frequency of sensory discharge is reduced through inhibition of CD73 and adenosine receptors. This is significant given that chronic mitochondrial dysfunction is linked to an increase in CB sensory activity in animal models of obstructive sleep apnea (Duchen & Biscoe, 1992; Buckler & Turner, 2013; Holmes et al., 2016). Additionally, a run-down in mitochondrial energy metabolism is thought to be a prerequisite for coupling hypoxia with type 1 cell stimulation (Duchen & Biscoe, 1992; Buckler & Turner, 2013; Holmes (Peng et al. 2003). Our results thus provide evidence that functional inhibition of CD73 reduces baseline and hypoxic discharge frequency as well as responses to stimuli associated with CB disease [Holmes A.P. et al., 2017].

α,β-methylene-ADP (AOPCP) was administered intravenously to suppress CD73 in vivo, which inhibited HVR and resulted in a less pronounced decrease in PaCO2 under hypoxia. These findings support in vitro studies and indicate that AOPCP successfully reduces CB hypoxia sensitivity in vivo. The acute phase of the HVR in rats has also been shown to be inhibited by the substance 8SPT in prior studies (Lee et al. 2005), and our data suggest that this is because adenosine is crucial for developing the CB's sensitivity to hypoxia [Holmes A.P. et al., 2017]. AOPCP additionally exacerbated the rise in FVC and decline in MABP while reducing the HR response to hypoxia. These findings are in line with CD73 inhibition-induced hypoxia's lower increase of sympathetic outflow to the heart and vascular system. Given the positive association between sympathetic outflow and CB chemodischarge during hypoxic stimulation, the decreased sympathetic activity is most likely the result of decreased CB activation. Additionally, this might be brought on by diminished CB hypoxic chemodischarge and respiratory drive, which again has the effect of blunting hyperventilation and lung stretch receptor activation. Pharmacological suppression of CB CD73 may provide a unique and significant method of lowering cardiovascular sympathetic output in CB-related disease if similar findings are confirmed in humans. (2017) [Holmes A.P. et al.]

Nevertheless, a considerable drop in PaCO2 revealed that AOPCP also induced a significant hyperventilation in normoxic settings. Additionally, it caused a rise in resting HR and a decrease in FVC in normoxia. It is known that CD73 is expressed in areas of the brain where it controls the production of adenosine (Chu et al. 2014). Additionally, central respiratory drive is decreased by A1 receptor activation (Montandon et al. 2007). We therefore propose, albeit speculatively, that hyperventilation under normoxic settings results from a decrease in adenosine concentration in the central respiratory brain areas when cerebral CD73 is inhibited. The intriguing idea that CD73 may possibly have a significant functional role in mediating central respiratory drive under normoxic settings is raised by these observations. According to these findings, it may be necessary to produce nucleotidase inhibitors that do not pass the blood-brain barrier or to specifically target the CB CD73 in order to lessen CB sensory activity [Holmes A.P. et al, 2017].

3. Ecto-5’-nucleotidase (CD73) and arteriogenesis

Adenosine can enhance angiogenesis, although it is unclear what part it plays in the unique process of arteriogenesis. According to earlier studies, mice lacking ecto-5’-nucleotidase (CD73) exhibit increased post-ischaemic monocyte adherence to the endothelium, which is regarded as a key initiator of arteriogenesis [Schrader J. et al, 2013].

Serial analysis of the dynamic changes in artery growth and metabolism during the process of arteriogenesis shows that, in our model of hindlimb ischaemia, the absence of CD73-derived adenosine significantly increases arteriogenesis but does not affect angiogenesis. 2013 [Schrader J. et al]

By stimulating the formation of collateral arteries and connecting arterial lesions that have undergone stenosis or occlusion with "natural bypass" channels, the modification of arteriogenesis has the potential to lead to new treatments for atherosclerotic obstructive disorders. Endothelial cells in collateral vessels experience increased fluid shear stress (FSS) as a result of hemodynamic changes brought on by artery obstruction, which causes them to become "activated." This is considered to be a crucial initial trigger for arteriogenesis, which is then anticipated to be followed by circulating monocyte attraction, which is assumed to be a prominent cellular participant in this intricate process. Numerous mediators have been found to support and maintain collateral remodeling, including monocyte attracting protein (MCP-1), granulocyte-monocyte-colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), fibroblast-growth factor (FGF-2) and tumour necrosis factor (TNF-).

Adenosine is a common nucleoside that is produced both inside and outside of cells by the stepwise dephosphorylation of ATP. It is known to be a pro-angiogenic agent that promotes capillary formation in hypoxic conditions. Since adenosine is also thought to stimulate the development of new blood vessels, we targeted disruption of the CD73/ecto-5’-nucleotidase to remove a significant source of external adenosine. Four cell-surface receptors (A1, A2A, A2B, and A3) that are known to promote endothelial migration, proliferation, and VEGF production are involved in adenosine signaling. Adenosine A2A receptors have recently been mentioned as being crucial in the remodeling of the pulmonary arteries during pulmonary hypertension. In a model of acute lung injury, it was further demonstrated that activation of A1 receptors could decrease leucocyte infiltration. Adenosine's anti-inflammatory properties are connected to the activation of A2A receptors on immune cells, which dramatically reduces the release of cytokines and chemokines. The extracellular adenine nucleotides ATP/ADP, which are converted into adenosine via CD39 (ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5’-nucleotidase), transmit signals via the more than nine known P2 receptors. Nucleotides are well known to affect the release of different cytokines like TNF- and interleukin (IL)-1 and to govern leucocyte adhesion and trafficking via the endothelium. Their role in the regulation of platelet function is well understood. It is crucial to understand that the biological half-life of extracellular ATP is influenced by CD39 activity, which along with CD73 controls whether ATP or adenosine receptors are preferentially activated [Schrader J. et al, 2013].

In a prior work, we found that ex vivo-perfused carotid arteries and increased leucocyte attachment to the endothelium during ischaemia-reperfusion are both seen in mouse mutants missing CD73. Using a mouse hindlimb ischaemia model, the current work investigated the involvement of CD73 in this process since monocyte adherence is known to be an important early step in arteriogenesis. Serial in vivo magnetic resonance angiography (MRA) measurements were used to track arteriogenesis. This method allowed for the sensitive quantification of luminal regions for interindividual comparison and the direct visualization of freshly generated vessels. Additionally, as a gauge for tissue recovery during ischaemia, we examined energy metabolism using 31P MR spectroscopy. Histology and immunohistochemistry were used to supplement the in vivo results [Schrader J. et al, 2013].

When big conduit arteries get blocked, the body responds in two different ways: angiogenesis and arteriogenesis. Angiogenesis defines capillary growth by sprouting from pre-existing vascular structures, whereas arteriogenesis

refers to the adaptive extension of pre-existing collateral arteries to circumvent arterial stenoses in response to haemodynamic changes. The current work shows that absence of CD73-derived adenosine increases arteriogenesis but has no effect on angiogenesis in the model of hindlimb ischaemia. Therefore, it appears that adenosine, which is produced by extracellular nucleotide catabolism on immunological and endothelial cells, is a key endogenous regulator of arteriogenesis [Schrader J. et al., 2013].

By overlapping slab acquisition, which reduced signal loss, the sensitivity for identifying changes in blood flow and consequently vessel size was further enhanced. The ability to measure HEP content (ATP, PCr) as a functional correlate in the damaged hindlimb muscle is another benefit of using MR methods to evaluate arteriogenesis. It was discovered via MRI and MRS that the CD73 mutant's increased arteriogenesis was connected to a quicker return of muscle HEP. This strongly implies that muscle energetics and function were greatly enhanced by increased blood flow and subsequently oxygen delivery. Therefore, it is most likely that the higher ATP tissue levels found in the CD73 mutant are a direct result of improved oxygen supply through increased arteriogenesis and do not correspond to variations in ecto-nucleotidase activities [Schrader J. et al, 2013].

After a primary supplying stem arterial becomes blocked, pre-existing collateral vessels remodel "outward," leading to artery expansion and higher conductance. Haemodynamic variables, such as an increase in stretch and FSS on endothelial cells, drive this process. By expressing MCP-1 and ICAM-1, FSS-activated endothelial cells do draw mononuclear cells that penetrate the vessel wall. It is widely known that monocytes and pro-inflammatory cytokines play a crucial part in adaptive arteriogenesis. More recently, it has been discovered that toll-like receptors 2 and 4 have a role in the development of adaptive collateral arteries, implying a connection to innate immunity. Additionally, the absence of HIF hydroxylases PHD2 avoids ischaemia via promoting arteriogenesis, perhaps by regulating the macrophage differentiation status. 2013 [Schrader J. et al]

The well-known ability of adenosine to reduce the pro-inflammatory responses of classically activated macrophages by Th1 cytokines is of special importance given the crucial role that monocytes and macrophages play in the process of arteriogenesis. The pro-inflammatory phenotype of CD73 mice, which includes increased leucocyte attachment to the endothelium following ischaemia-reperfusion experiments, elevated macrophage content following wire-induced injury to the carotid arteries, and increased endothelial NF-B activation with up-regulation of endothelial adhesion protein, including VCAM-1, are all characteristics of these mice. Additionally, it was shown that CD73 is a crucial modulator of vascular barrier function during hypoxia, and that its absence causes fulminant vascular leakage. In the current work, 3 days after the production of hindlimb ischaemia, we noticed increased monocyte invasion in CD73 mice. Adenosine reduces the production of VCAM-1 by preventing the release of IL-6 and IL-8. Integrin 41 (also known as very late antigen-4, VLA4) and VCAM-1 interact to increase monocyte adherence. Through a cAMP/PKA-mediated mechanism that improves endothelial barrier function, reduces vascular permeability by fortifying intercellular junctions, and prevents TNF- production, stimulation of A2B receptors also decreases VLA4 expression. By directly interacting with CD73, adherent leucocytes can lower extracellular adenosine and so encourage leucocyte transmigration. Additionally, CD73 depletion increases the expression of ICAM-1, VCAM-1, and E-selectin, promotes NF-B translocation, and alters endothelial cell morphology with altered actin cytoskeletal organization, which is similar to the phenotype seen under the treatment with TNF-. These findings were obtained from CD73 knockdown experiments using RNAi. Therefore, adhering monocyte diapedesis to initiate arteriogenesis was very likely promoted by the absence of CD73-derived adenosine [Schrader J. et al, 2013].

The ability of adenosine to reduce inflammation is widely recognized, and in 1986, adenosine was shown to suppress neutrophil activation. Due to the enormous phenotypic plasticity of macrophages, two populations—classically activated M1 and alternatively activated M2—have been identified. Adenosine, which is mediated by both A2A and A2B receptors, suppresses TNF-, IL-6, and IL-12 production and increases IL-10 and VEGF during classical stimulation with LPS. Adenosine has recently been shown to support alternate macrophage activation via the A2A and A2B receptors. It is unclear if CD73-derived adenosine influences arteriogenesis by acting on M1 and M2 macrophages [Schrader J. et al, 2013].

Adenosine may encourage angiogenesis in tissue that is ischemic, according to a number of pieces of data. In vitro investigations have revealed that adenosine also promotes endothelial cell proliferation, migration, and ensuing network creation. The release of VEGF from monocytes and angiogenesis are both encouraged by the activation of A1 receptors. Thus, it was first unexpected that angiogenesis, as measured by day 7 measurement of capillary density, was minimal in WT mice and did not demonstrate a significant difference between the two genotypes. It is probable that arteriogenesis diminished tissue hypoxia and consequently decreased the degree of angiogenesis because hypoxia is a significant trigger for angiogenesis. Similar to this, Deindl et al. observed that in a rabbit hindlimb ischaemia model, neither HIF-1-mRNA nor the HIF-controlled VEGF gene expression was significantly up-regulated. The improved arteriogenesis in CD73-deficient mice further reduced tissue hypoxia, which may account for the quicker recovery of HEP. Therefore, it is likely that the absence of CD73's effects on angiogenesis in our model is caused by CD73's role in upstream arteriogenesis, which reduced the severity of distal ischaemia by boosting blood flow [Schrader J. et al, 2013].

4. Ecto-5'-nucleotidase (CD73) and Lung Cancer

Adenosine is a crucial regulator of respiratory function, and adenosine receptor signaling manages the homeostasis of airway epithelial cells, safeguards tissue barrier function through activity on endothelial cells, and controls the release of inflammatory mediators from a variety of immune cell types. Vascular leakage in response to normobaric hypoxia was one of the early phenotypes observed in the global Nt5e mice, but it was most evident in the lung. Recent studies have highlighted the roles played by CD73 in lung inflammation and fibrosis in addition to its role as a crucial regulator of lung injury in response to variations in oxygen. In 2019, Minor M. et al.

The primary regulator of adenosine synthesis on airway surfaces, along with TNAP, is CD73. Human pneumocytes, which display both cytoplasmic and membrane distribution, exhibit significant levels of CD73 expression, according to the Human Protein Atlas. The majority of extracellular adenosine produced on the mucosal and serosal surfaces of human airway epithelia is a result of CD73 activity. Ion transport and appropriate cilia beating frequency are both governed by extracellular adenosine. Adenosine produced by CD73 by epithelial cells also plays a protective effect during acute lung injury by lowering endothelial permeability. As a result, CD73 activity on airway epithelial cells controls mucociliary clearance and promotes lung disease defense. In 2019, Minor M. et al.

Adenosine receptor agonists and the delivery of soluble CD73 only partially prevent hypoxia-induced vascular leakage in Nt5e mice, suggesting that CD73 may have other nonenzymatic activities that are damaged in the Nt5e mouse. An open-label clinical experiment assessing the efficiency of intravenous IFN-1 on acute respiratory distress syndrome mortality was justified using subsequent research that demonstrated CD73 is a crucial target of IFN-mediated protection against vascular leakage in the lungs. The latter study showed that IFN-1 therapy decreased 28-day mortality and that CD73 was dramatically elevated ex vivo in peribronchiolar arteries of cultured human lung tissue (8 percent mortality in treated patients vs. 32 percent mortality in the control untreated group). These results highlight the potential for CD73-mediated protection during hypoxic lung injury, even if it was targeted by an indirect, cytokine-dependent mechanism [Minor M. et al, 2019].

During hyperoxic lung injury, CD73 is also significantly increased, and Nt5e mice exhibit more severe pulmonary edema. The latter impact, which is phenocopied in A2AR animals, is linked to loss of barrier function when adenosine synthesis is diminished. Elevating CD73-generated adenosine is required to reduce aberrant alveolar formation in a neonatal mouse model of hyperoxia. In particular, newborn mice exposed to severe hyperoxia (95 percent O2) have markedly impaired alveolar development, which is made worse in Nt5e animals, and results in 100% mortality by day 11 of exposure (in contrast to 44 percent mortality in the WT mice). As seen by greater lung infiltration of macrophages and lymphocytes in a less severe (70 percent O2) condition, Nt5e animals had higher inflammation levels than WT mice. In humans, bronchopulmonary dysplasia in preterm newborns can be encouraged by hyperoxic settings (such as oxygen supplementation). It's interesting that coffee, a nonselective adenosine receptor antagonist, lowers the incidence of bronchopulmonary dysplasia in newborn humans. The

apparent discrepancy between preclinical results in mice and humans may be the result of changes in embryonic stages, general species differences, or a protective function of CD73 that is compromised in the Nt5e model but is present in other species. To better understand whether processes of CD73 are preserved across species, rigorous integration of data utilizing animal models must be considered alongside suitable human-derived model systems [Minor M. et al, 2019].

In the presence of lung inflammation, adenosine is created as a defensive response, and CD73 is crucial to this mechanism, as was first demonstrated using a bleomycin model of inflammatory lung injury. Exogenous nucleotidase was administered intravenously to Nt5e mice after bleomycin treatment to reduce the severity of the lung fibrosis, increased inflammation, and increased collagen formation. The increased weight loss and inflammation that Nt5e animals experienced in response to intratracheal lipopolysaccharide treatment, as well as the considerable transcriptional upregulation of TNF-, IL-1, and IL-6, provide additional evidence for the anti-inflammatory activity of CD73. These effects were linked to reduced regulatory T cell adenosine synthesis, and injection of soluble CD73 partially reversed the inflammatory phenotype. Pneumococcus infection is also affected by the anti-inflammatory characteristics of CD73-produced adenosine, which is mediated through the mobilization of polymorphonuclear leukocytes (PMNs) to reduce bacterial burden. Even if an early PMN response is advantageous, having PMNs in the lungs for an extended period of time is harmful. Pneumococcus infection led to the production of extracellular adenosine by CD73, which prevented later-stage PMN from crossing the endothelium and entering the lungs. More PMNs transmigrated when CD73 was absent, but they were unable to reduce the bacterial burden. As a result, CD73 both restricts PMN migration and increases their ability to kill pneumococcus bacteria. In 2019, Minor M. et al.

In contrast to the anti-inflammatory and antifibrotic properties mentioned above, CD73 is a factor that promotes radiation-induced lung fibrosis. In mice that underwent 15-Gy whole thorax irradiation 25–30 wk after the therapy, when substantial fibrosis was already present, CD73 activity was enhanced thrice. Adenosine concentration in the bronchoalveolar lavage fluid significantly increased along with increased immune cell infiltration into the tissue and raised CD73 activity. The development of epithelial injury and fibrosis in response to the same radiation therapy, however, was greatly inhibited in CD73 animals or in WT mice treated with the anti-CD73 monoclonal antibody TY/23, despite identical numbers of infiltrating leukocytes. It was postulated that long-term, persistent rise of pulmonary adenosine levels promotes pathogenic tissue remodeling, albeit the precise molecular pathways remain unclear. In 2019, Minor M. et al.

Although immunotherapy and tyrosine kinase inhibitor therapy have considerably improved the management of lung cancer, many patients do not benefit from them or develop resistance to it, emphasizing the need for new therapies. Non-small cell lung cancer (NSCLC), including those containing the RAS- or RTK (EGFR, EML4-ALK) oncogenes, frequently exhibits elevated CD73 expression. The epithelial-mesenchymal transition transcriptome signature and the immune-tolerant tumor microenvironment, which are becoming increasingly important for disease progression and therapeutic resistance, are closely enriched with CD73 expression. While neither the naked CD73 antibody nor conventional chemotherapy produced any intratumoral increase of proinflammatory macrophages or activated dendritic cells (DC), treatment with CD73-ADC did. Studies using systems derived from human PBMCs demonstrated that CD73-ADC is fully functional in protecting effector T cells and stimulating DCs, resulting in a dual advantage of eradicating CD73-high tumors and enhancing the immune response to cancer. These findings call for clinical testing of CD73-targeted antibodies and ADC in the management of advanced lung cancer. 2020 [Yu K. et al.]

There is proof that an acute CD73-dependent rise in adenosine mostly serves tissue protective activities in pre-clinical research in models of injury-induced sterile inflammation. Here, the self-termination of TLR responses in macrophages by purinergic signaling may be a factor in the outcomes seen.

Contrarily, chronically elevated adenosine levels can encourage pathologic remodeling processes in different tissues that result in the development of fibrosis, as in the case of genetic adenosine-degrading enzyme ADA deficiency or prolonged administration of the chemotherapeutic agent bleomycin (BLM). Alternately activated myeloid cells are thought to be responsible for the pathogenic effects of BLM-induced chronic adenosine buildup in the lung. 2019 [Jendrossek V.]

Only one individual's research has addressed the significance of purinergic signaling for radiation-induced late-onset deleterious effects in the lung; other researchers focused on the skin. Our research showed that chronically elevated CD73/adenosine signaling has a pathological function in the irradiated lungs of C57BL/6 mice, most likely by increasing or amplifying profibrotic signaling cascades. Adenosine levels in the bronchioalveolar lavage fluid gradually increased in response to pathologic signaling, which involved a time-dependent increase in the expression and activity of CD73 in lung tissue that could be restricted to resident CD45+ immune cells (CD4+ T cells, including T-reg, and alveolar macrophages). C57BL/6 mice with a CD73 knockout did not accumulate high levels of adenosine in response to WTI, leading to Treatment of irradiated C57BL/6 mice with either pegylated ADA (PEG-ADA) to catabolize adenosine or the CD73 monoclonal antibody (mAb) TY/23 as of week 16 post-irradiation produced a similar protective effect. 2019 [Jendrossek V.]

Together, the gradual activation of CD73/adenosine signaling in the irradiated lung environment encourages the buildup of immunosuppressive innate and adaptive immune cell types, such as T-reg and M2-like macrophages, and supports pro-fibrotic cross-talk between damaged resident cells and infiltrating immune cells. Thus, radiation-induced lung fibrosis as a late normal tissue consequence is amplified by CD73/adenosine signaling. Adenosine also contributed to radiation-induced skin fibrosis, supporting our findings. However, in this case, the pro-fibrotic effects were mostly due to T-cell infiltrates and signaling via ADORA2A, without the involvement of alternatively activated macrophages. 2019 [Jendrossek V.]

Immune cell infiltrates in human cancers display distinct changes in cell kinds and numbers, not only intratumorally but also between people and other tumor forms, according to analyses of patient biopsies. It was surprising to find that the distribution and kind of infiltrating immune cells had predictive significance; for instance, the presence of infiltrating T cells was mostly associated with a positive clinical result. These results were corroborated by additional pre-clinical and clinical research demonstrating the immunogenicity of tumors, which can be high or weak. Additionally, immunogenicity levels were positively connected with decreased tumor development and improved survival of tumor-bearing mice in response to immunotherapy, suggesting that the immunological status may be used as a predictor of treatment outcomes. 2019 [Jendrossek V.]

While relapse following chemoradiotherapy and early recurrence connected to myeloid cell infiltration, high numbers of tumor-infiltrating cytotoxic lymphocytes were also prognostic for the response of head and neck cancer patients to radiation therapies. In pre-clinical models, poor tumor response to radiation and tumor relapse have also been linked to local or systemic elevations in T-reg, high numbers of tumor-associated macrophages, or recruitment of CD11b+ myeloid cells. It has been demonstrated that the combination with cancer vaccines, immune checkpoint blockade, or reduction of CD11b+ cell recruitment can enhance the outcome of radiotherapy as a proof of concept for the synergistic interaction of radiotherapy and immunotherapy. 2019 [Jendrossek V.]

It is noteworthy that cancers manipulate the purinergic CD39/CD73/adenosine system to alter the immunological milieu in the tumor microenvironment on many levels: For instance, in hypoxic malignancies, tumor cells and tumor-associated T-reg use CD73-dependent adenosine production to suppress intratumoral immune responses. The re-direction of the immune response involved communication via stimulation of the ADORA2A on effector T cells and repression of T cell effector capabilities through CD73-dependent synthesis of extracellular adenosine by CD39+/CD73+ T-reg. Thus, through inhibiting CD8+ T lymphocytes, adenosine and ADORA2A contribute to the development of an immunosuppressive tumor microenvironment. Other pre-clinical models had similarly shown

an adenosine-dependent inhibition of immunosurveillance via IFN-, NK cells, and CD8+ T cells. Last but not least, the development of immunosuppressive myeloid cells, including as myeloid-derived suppressor cells, M2-like macrophages, and maybe N2-like neutrophils, contributed to the development of an immunosuppressive tumor microenvironment. The following reviews [Jendrossek V., 2019] provide more information regarding the varied effects of CD73 and adenosine on cells from the innate and adaptive immune systems in the tumor microenvironment and the involved ADOR receptors.

The CD73/adenosine system also promotes tumor spread, tumor neovascularization, and resistance to chemotherapy, even if some of these effects may also be a result of the regulation of immune cell types in the tumor microenvironment. 2019 [Jendrossek V.]

Interestingly, after receiving intravenous injections of B16F10 or TRAMP-C1 cells, CD73 animals also showed a reduction in lung metastases, indicating that host CD73 also promotes metastasis. Following injection of 4T1.2 or TRAMP-C1 tumor cells, therapy with an anti-CD73 mAb (TY/23) significantly reduced the lung metastases. However, metastasis formation was suppressed in both immunocompetent and SCID animals, and it was discovered that CD8+ T cells and NK cells had no effect on this. By doing so, the authors identified a role for CD73+ non-hematopoietic host cells—possibly endothelial cells—in the development of metastases. They were also able to connect the pro-metastatic effect to the activation of ADORA2B, which signals the tumor's extracellular adenosine. 2019 [Jendrossek V.]

In additional research, adenosine produced by tumors attracted myeloid cells and encouraged their development into tumor-associated macrophages (TAM), which increase adenosine-dependent tumor-immune escape. In vitro exposure to adenosine boosted alternative macrophage activation and improved macrophage immunosuppressive responses to danger signals, especially if triggered in the presence of TLR ligands. Intriguingly, tumor-derived CD73-dependent adenosine promoted growth, neovascularization, and metastasis of subcutaneous B16F10 melanoma tumors and this was linked to infiltration and polarization of macrophages: When compared to untreated B16F10 wildtype tumors on CD73 m, genetic or pharmacologic inhibition of CD73 on the B16F10 melanoma cells significantly reduced the number of tumor Granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon gamma (IFN-) production of pro-inflammatory cytokines was down-regulated and anti-inflammatory/pro-angiogenic cytokines were expressed more abundantly in CD73+ B16F10 wildtype tumor lysates cultured on CD73 mice (IL-4, IL-10, IL-13, M-CSF). Less MMR+ macrophages were detected inside the tumor, even though the quantity of infiltrating macrophages did not alter in CD73+ B16F10 WT tumors on CD73 mice. The number of invading macrophages was only decreased by pharmacological CD73 inhibition or CD73 knockdown in the tumor host. The findings suggest that CD73 plays a part in the activation and polarization of macrophages that aid in the development of tumors. Additionally, it was demonstrated that ADORA1, ADORA2A, and ADORA3 were necessary for the activation and recruitment of tumor-infiltrating macrophages. 2019 [Jendrossek V.]

When considered collectively, loss of CD73/adenosine signaling improves tumor immunity while CD73-dependent adenosine from host and tumor cells contributes to the promotion of tumor growth by, among other things, boosting tumor immune evasion. CD73/adenosine has developed into an appealing therapeutic target in (immuno)-oncology, as expertly outlined in a recent review from Allard et al. Inhibitors of CD73 and adenosine are currently being tested in a number of early-stage clinical trials to determine whether they have the ability to treat tumor immunity and growth. The discovery of the specific ADOR implicated in increasing tumor immune escape will provide other avenues for therapeutic intervention in addition to the direct suppression of CD73. 2019 [Jendrossek V.]

At least in pre-clinical models of breast and colon cancer, co-inhibition of adenosine signaling via CD73 and ADORA2A elicited greater anti-tumor immune responses than solo therapies. Improved immune cell infiltration, DC priming, and CD8+ T cell growth were all linked to these benefits. Young et al. also noted greater tumor

development delay in CD73/ADORA2A double deletion mice, which is consistent with these findings. Studies with the human monoclonal anti-CD73 antibody MEDI9447, which is presently in Phase I clinical trials, also demonstrated high efficacy in inhibiting CD73 in vitro and potent inhibition of pre-clinical syngeneic tumor models in vivo, as well as additive activity when combined with immune checkpoint inhibitors. It's interesting to note that MEDI9447 effectively altered the tumor microenvironment, notably changing the proportions of both CD8+ effector T cells and activated macrophages. 2019 [Jendrossek V.]

Significant research is also being paid to the immunosuppressive effects of CD73 and adenosine in the microenvironment of existing malignancies as an intriguing target for combination treatment approaches, particularly with immunotherapy. In this situation, CD73 knockdown improved the effectiveness of immunotherapy using PD-1 or CTLA4 in pre-clinical mice. Improved T cell effector activity and decreased CD73 expression on tumor-infiltrating lymphocytes were two outcomes of the combined therapy that depended on interferon gamma (IFN-) and perforin. ADORA2A was therapeutically inhibited to improve effector function, alter T cell co-inhibitory receptor expression, and immune checkpoint blockade and adoptive cell treatment efficacy in mouse cancer models. 2019 [Jendrossek V.]

We won't go into further depth about CD73 and adenosine inhibition here because the main goal of this review is to emphasize their therapeutic potential for enhancing the therapeutic benefit of radiation. Please refer to reviews that go into more detail on the purinergic pathway's therapeutic potential in immunotherapy for further information.

As an alternative, the therapeutic potential of combining radiation or radioimmunotherapy with CD73/adenosine-inhibition in cancer has been emphasized as an alluring strategy, but solid data are still lacking at this time. Pre-clinical research, including experiments in our own lab, is currently being conducted to explore such strategies. 2019 [Jendrossek V.]

According to several pre-clinical investigations on the function of CD39 in cancer, mice lacking the CD39 gene are more resistant to the spread of melanoma and colorectal cancer models. Similar to this, LLC and B16F10 tumor development and lung metastasis were inhibited in a background with CD39 deficiency. The inhibition of NK cell-mediated antitumor activity and angiogenesis were both aided by CD39 expression. According to these findings, CD39 overexpression increased the potential for metastatic spread in pre-clinical mice, but CD39 pharmacological suppression decreased metastasis and improved antitumor immunity. A noteworthy point is that clinical evidence suggests a link between high CD39 expression and a bad prognosis, suggesting that CD39 may be yet another interesting target for cancer treatment. However, compared to CD73, CD39 is significantly less studied as a cancer target, highlighting the need for additional pre-clinical research. 2019 [Jendrossek V.]

There is currently a dearth of information about CD73 and adenosine's function in lung cancer. Here, it was discovered that CD73 was expressed on tumor cells, tumor-promoting mesenchymal stromal cells, and myeloid-derived suppressor cells, respectively, in tumor tissue from NSCLC patients. In tumor tissues and peripheral blood of NSCLC patients, tumor-derived TGF- promoted CD39 and CD73 expression in CD11b+CD33+ MDSC, suppressing T cell and NK cell activity and shielding tumor cells from the cytotoxic effects of chemotherapy. 2019 [Jendrossek V.]

Additionally, there is ongoing debate concerning the predictive significance of high CD73 expression for lung cancer patients' survival: While one study found a link between high levels of the CD73 gene and better overall survival in NSCLC patients, an other study found that high levels of the CD73 protein are a separate prognostic factor for worse overall survival and shorter recurrence free survival in NSCLC. It's interesting to note that in the same study, high ADORA2A gene expression was a reliable indicator of a good chance of overall survival. The favorable link between high CD73 gene expression and higher overall survival of NSCLC patients was validated by individual in silico analysis of publically accessible datasets for CD73 gene expression in lung cancer. It should

be noted that the association to a better overall survival was eliminated if patients who had received radiotherapy were not included in the analysis. Additionally, the in silico analysis showed that lung cancer patients with high ADORA1, ADORA2A, and ADORA2B gene expression had a worse overall survival rate. Once more, the conclusions provided regarding the prognostic significance of ADORA2A utilizing immunohistochemistry data were inconclusive. Given that CD73 expression in tumor samples was found to be highly heterogeneous, the disparity in the aforementioned findings may be related to the use of gene expression analyses as opposed to immunohistochemistry data. The difficulty in gathering representative gene expression data may be due to the variety of CD73 protein expression in various tumor regions, which may be related to variable tumor oxygenation. Although such investigations are under progress, CD39 inhibitors have not yet been tested in clinical trials for cancer patients [Jendrossek V., 2019].

When combined, adenosine produced via the CD39/CD73 axis or released during an inflammatory environment will have an effect on the immunological profile of lung tumors, likely by reducing T cell immunity and boosting immunosuppressive and tumor-promoting lymphoid and myeloid immune cell phenotypes. Combining CD73/adenosine signaling modulation with radiotherapy and maybe radioimmunotherapy is a promising approach to inhibit tumor growth, enhance antitumor immune responses, and prevent therapeutic escape. Conversely, the pathologic role of the radiation-induced increase in CD73/adenosine signaling in promoting chronic inflammation and fibrosis in the normal lung tissue strongly suggests that pharmacologic inhibition of CD73/adenosine offers the opportunity to widen the therapeutic window by reducing radiation-induced lung toxicity, particularly in CD73-rich thoracic tumors with a high risk for CD73-dependent normal tissue toxicity. 2019 [Jendrossek V.]

We anticipate that inhibiting CD73/adenosine signaling will limit lung toxicity during thoracic irradiation without protecting the tumor or even restore anti-tumor immunity when used during therapeutic irradiation of adenosine-rich tumors with high radioresistance such as NS. Targeting the CD73/adenosine pathway or the involved receptors may therefore provide a clear therapeutic gain in the treatment of lung cancer and other CD73/aden To prevent the lung from suffering a loss in critical function brought on by inflammation, resident and immune cells must be tightly controlled in their pro- and anti-inflammatory functions. To reduce inflammation-induced collateral damage to normal tissue following radiotherapy, immunosuppressive T-reg are one example of a protective response. Therefore, thorough confirmation of possible problems in normal tissue will be necessary for pharmaceutical techniques targeting the CD73/adenosine pathway in combination with radiation or combined radioimmunotherapy. By disabling the protective signals mediated by different ADORAs, such problems may include increased inflammation or autoimmunity, especially during acute illness stages. Additionally, while developing combination therapies for therapeutic intervention, it is important to take into account the dual impacts of acute and chronic CD73 activation as well as the spatiotemporal heterogeneity of CD73 and ADOR expression in normal and malignant tissues [Jendrossek V., 2019].

5. CD73 and Adenosine in Lung Transplantation

Bronchiolitis obliterans syndrome (BOS), which is characterized by progressive airflow obstruction and functional decline, is a manifestation of chronic pulmonary allograft malfunction. Following lung transplantation, BOS is a serious complication that lowers long-term survival. Acute rejection episodes, CMV infection, and ischemia-reperfusion damage are independent predictors of progressive BOS. In 2014, [Dwyer K.M. et al.]

In order to prevent inflammation from spreading quickly after lung transplantation, CD73-dependent adenosine synthesis is crucial. In fact, after one week, tracheal allografts given to recipient animals lacking CD73 showed more inflammation. Increased CD3+ T cell infiltration and higher levels of the Th1 cytokines IL-2 and IFN- caused by the inflammatory infiltrate led to luminal constriction. A2AR mRNA

expression was clearly markedly upregulated in the WT allografts, to the point that CD73-deficient recipients were treated with an A2AR agonist to restore the allograft. In fact, if given before ischemia or during reperfusion, A2AR activation potently reduces lung IRI In 2014, [Dwyer K.M. et al.]

In a blood-perfused rabbit lung model that underwent ischemia and then received therapy with the particular A2AR agonist ATL313, inflammation and pulmonary edema were maximally reduced, and lung function was optimized. This impact was eliminated when ischemia and an A2AR inhibitor were coupled. Additionally, A2AR activation reduces cardiac dysfunction that coexists with pulmonary IRI.

In mice, A2AR activation improves lung function by reducing CD4+ T cell and neutrophil infiltration as well as inflammatory cytokines like TNF, IL-17, MCP-1, MIP-1, and RANTES.

The cold preservation phase of human lung transplantation reduces cellular metabolism and limits ischemia injury; nevertheless, prolonged cold storage times increase the likelihood of delayed graft function. Following cold preservation and transplantation, A2AR activation reduces the inflammatory response and maintains pulmonary function. Reece et al. showed that treatment of the recipient pig with A2AR agonist commencing 10 min before and continuing for the first 3 h of reperfusion improved outcome using a porcine transplant model. In this model, the pig lung underwent 4 hours of reperfusion after 6 hours of cold ischemia. Animals given the A2AR agonist ATL-146e intravenously showed enhanced oxygenation with intact CO2 levels and acid-base balance. The A2AR agonist treatment group also had lower measured pulmonary artery pressures and pulmonary vascular resistance. Because there was less pulmonary infiltration in the therapy group, the overall lung injury score was much lower [Dwyer K.M. et al., 2014].

Less edema, a better oxygenation index, a lower mean airway pressure, and lower levels of IFN, IL-1, IL-6, and IL-18 were seen in the treated lungs, indicating that A2AR agonist supplementation may further improve lung function before transplantation. BOS is facilitated by recurrent episodes of acute allograft rejection. Modifying the alloimmune response requires A2AR signaling: By preventing inflammatory islet damage during the peri-transplant period, A2AR activation decreases skin allograft rejection and enhances the survival and functional engraftment of transplanted islets [Dwyer K.M. et al, 2014].

7. Functions in the Digestive system:

1. ROLE of CD73 IN GASTROENTEROLOGY

Radiation therapy's (RT) anti-tumor actions are heavily reliant on the health of the host immune system. An essential immunological checkpoint molecule, adenosine has potent immunosuppressive effects [Tsukui H. et al, 2020]. The expression of CD73 in the highly metastatic murine colon carcinoma LuM-1 is considerably increased following RT. Within two weeks after subcutaneous (sc) transfer of LuM-1, Balb/c mice exhibited microscopic lung metastases and macroscopic sc tumors. After RT, the sc tumor's adenosine levels rose. The growth of lung metastases that were protected from RT was unaffected by selective RT (4Gyx3), whereas the growth of the irradiated sc tumor was reduced [Boucher C. et al, 2003]. Anti-CD73 antibody (200 g/6) administered

intraperitoneally alone had no anticancer effects. However, when used in the same protocol as RT, anti-CD73 antibody further inhibited the growth of lung metastases and slowed the growth of sc tumors, likely due to abscopal effects. Splenocytes from animals treated with the RT+ CD73 antibody produced more IFN- and were more cytotoxic to LuM-1 than control splenocytes. High expression of CD73 in residual tumor cells and/or stroma is substantially related with a worse outcome, according to immunohistochemical investigations of irradiated human rectal cancer [Tsukui H. et al, 2020].

These findings imply that adenosine is crucial to the anti-tumor effects of radiotherapy and that blocking the CD73/adenosine axis may improve the prognosis of patients with locally progressed rectal cancer by enhancing the anti-tumor effects of radiotherapy.

Neoadjuvant radiation therapy (RT) is now accepted as the gold standard treatment for locally advanced RC all over the world because it can downstage locally advanced rectal cancer (RC), which leads to a lower rate of postoperative local recurrences. Recent research has demonstrated that combining RT with fluorouracil-based chemotherapy improves the locoregional control rate while not significantly increasing side effects. To increase the effectiveness and tolerance of RT, various radiosensitizers have recently been tested in clinical studies [Tsukui H. et al, 2020].

Commensal bacteria seem to be a significant source of intestinal luminal ATP in the gastrointestinal tract, indicating that the CD39/CD73 pathway may play a significant regulatory role in maintaining homeostasis while regulating infections. Recent studies reveal that primed uncommitted Th17 and T follicular helper cells also express CD73, raising the question of how this pathway controls immunity vs. tolerance in vivo even though CD39/CD73 expression was first noted on regulatory T cells [Oldenhove G. et al, 2015].

Although it has been assumed that DNA double-strand breaks or the induction of apoptosis are the key mechanisms for reducing tumor size, host immunological responses are equally crucial. It is generally accepted that RT causes temporary immunosuppression. However, numerous investigations have demonstrated that tumor cells that have died or are in the process of dying as a result of RT may still be able to convey tumor-associated antigens to host immune cells and elicit innate and adaptive immune responses. This results in the so-called abscopal effect, which causes tumors outside the irradiation field to regress in addition to increasing the lethal effect on tumor cells immediately exposed to RT [Tsukui H. et al, 2020].

Numerous studies have been carried out to determine the effectiveness of combination RT and immunotherapy because to the recent significant advancements in the understanding of immune checkpoint molecules. Pre-clinical research has shown that administering CTLA-4 and PD-1 antibodies at the same time as RT increases its anti-tumor effects. Clinical studies have revealed that RT and recently licensed antibody preparations against PD-1 and CTLA-4 have synergistic benefits. Benefits from combined modality therapy haven't been proven in other clinical research, though. As a result, it is still unclear what the ideal RT dose or RT fractionation should be, as well as what kind of agents should be used to maximize the response to RT [Tsukui H. et al, 2020].

An essential endogenous regulator of the innate and adaptive immune systems is adenosine. Through the A2A receptor, adenosine effectively inhibits immune cells and is essential for the preservation of homeostasis in a variety of organs. When the ectoenzymes ectoapyrase (CD39) and 5'ectonucleotidase work together, adenosine is either released from stressed or wounded cells or synthesized from extracellular adenine nucleotides (ATP, ADP, and AMP, or adenosine monophosphate) (CD73). The hydrolysis of ATP/ADP to AMP is catalyzed by CD39, and the conversion of AMP to adenosine by CD73 is thought to be the rate-limiting step in the synthesis of adenosine. As an immunostimulatory signal, ATP is one of the damage-associated molecular patterns (DAMPs). Balance between ATP and adenosine is thought to be essential for the local immunological response because adenosine, in contrast, has potent immunosuppressive effects [Tsukui H. et al, 2020].

Adenosine generated by the enzymatic activity of CD73 promotes metastasis and the survival of tumor cells through immunosuppression, as evidenced by the fact that malignant cells frequently express CD73 and that high CD73 expression in tumor tissue has been linked to poor clinical outcomes. In fact, numerous pre-clinical investigations have demonstrated that blocking the CD73/adenosine axis can slow the growth of tumors. These findings imply that a novel therapeutic approach to inhibit tumor growth may involve altering the amounts of adenosine in the tumor microenvironment. a mouse model of spontaneous lung metastases and tissue samples from patients with RC were used to examine the function of the CD73/adenosine axis on the tumor response to local RT [Tsukui H. et al, 2020].

RT has been routinely utilized to treat solid tumors, either with the goal of curing them or as a palliative measure. Recent clinical and pre-clinical investigations have revealed that the addition of immune checkpoint inhibition can dramatically improve tumor responses to RT. Due to its potent immunosuppressive effects, adenosine is increasingly recognized as a crucial metabolic immunological checkpoint molecule. Attention is being drawn to a potential type of immunotherapy that could be used in conjunction with RT: inhibition of the CD73/adenosine axis. However, it is unknown how adenosine level manipulation impacts RT results [Tsukui H. et al., 2020].

Th cells in human blood or stomach tissue that exhibit T-reg markers express CD39 and CD73 at much higher levels. In addition, relative to peripheral T cells, there is an even higher proportion of Th cells that resemble active Th cells and T-reg. Murine Th cells that express CD73 interact with the 5'-AMP substrate to produce enough adenosine to inhibit the function of effector Th cells. The finding that T-reg from CD73 mice failed to prevent gastritis illustrated the significance of CD73 on T-reg. It is crucial to understand how CD73 controls immunological and inflammatory responses and applies this idea to human Th cells in both systemic and mucosal regions (Alam M.S. et al., 2009). A highly metastatic clone of colon26 called LuM-1 has considerable levels of CD73 expression, which was further increased by radiation both in vitro and in vivo. Previous research has demonstrated that RT-associated proinflammatory cytokines and hypoxia both increase the expression of the CD73 gene. According to studies, RT increases the expression of CD73 in immune cells as well as cancer cells from the esophagus and bladder. It is probable that overexpression of CD73 leads to high amounts of adenosine accumulating in irradiated tumor tissue because following RT huge numbers of adenosine precursors are expected to be released into the extracellular space from injured cells [Tsukui H. et al, 2020].

Due to its low molecular weight, high polarity, and brief half-life as a result of enzymatic breakdown, accurate detection of tissue adenosine levels is difficult. Extracellular adenosine levels in solid tumors were found to be 50–100 M in previous investigations employing reversed phase high pressure liquid chromatography. These levels are higher than those in normal tissue and are sufficient to block local antitumor immune responses. Later, levels of inosine, a stable adenosine metabolite, were raised. These findings imply that adenosine levels are kept elevated, at least for hours, in the microenvironment of radiotreated tumors, which may reduce the anti-tumor immune response induced by RT [Tsukui H. et al., 2020]. In contrast to RT, anti-CD73 antibody did not inhibit the growth of sc LuM-1 tumors when given alone. In contrast to tumor growth in isotype control treated animals, antibody treatment further inhibited the growth of irradiation tumors when paired with RT. The amount of metastases in the unirradiated lungs was considerably reduced by anti-CD73 antibody. In the lungs of 50% of the mice given anti-CD73 together with RT, no metastases were seen. It is hypothesized that the combination of RT and anti-CD73 antibody elicits a systemic immune response that destroys lung tumor cells because microscopic metastases were present at the time of treatment. Splenocytes from mice given RT plus an anti-CD73 antibody produced more IFN- and were more cytotoxic to autologous LuM-1 in a test tube. These findings imply that the abscopal effects of RT can be caused by the anti-CD73 antibody, which may be partially ascribed to T cells that have been driven by RT-induced tumor-associated antigen [Tsukui H. et al, 2020].

Lung metastases may be slowed in growth by CD73 inhibition via immune-independent mechanisms. But given that anti-CD73 mAb did not significantly suppress lung metastases in vivo when used alone in this investigation,

it appears improbable. In actuality, CD73 mAb treatment had no impact on the in vitro migration and proliferation of LuM-1 cells [Tsukui H. et al, 2020].

In surgically excised human RC after CRT, immunostaining investigations revealed that CD73 was expressed in both residual tumor cells and/or stroma. Although each patient's expression pattern is unique, high CD73 expression was linked to a poor prognosis and a higher incidence of distant recurrence, which is in line with earlier research on non-irradiated tumors. Since concurrent chemotherapy increased CD73 expression in epithelial ovarian cancer and CD73 inhibition increased treatment efficacy, this may be partially attributed to the chemotherapy itself. However, in line with the findings of the murine studies, it has been hypothesized that elevated adenosine levels, caused by enhanced CD73 in irradiated tumor tissue, may impair systemic immune responses, which may be causally related to the development of micrometastases in distant organs in humans [Tsukui H. et al, 2020].

Radio-immunotherapy (RT) may be a successful therapeutic therapy for patients with advanced cancer since there is mounting evidence that it might induce in situ tumor vaccination by exposing tumor specific neoantigens to the host innate immune system. However, there are significant obstacles to comprehending the contradictory nature of RT-induced effects on the immune system. This is the first evidence to suggest that the anti-tumor response in irradiated tumors may be diminished by adenosine, which is recovered by effective CD73 inhibition. There has already been a phase 1 clinical trial using anti-CD73 mAb. Clinically, anti-CD73 mAb and RT are recognized as a promising preoperative therapy for patients with locally advanced RC [Tsukui H. et al., 2020].

Independent of ICS or TNM stage, elevated CD73 expression in tumor cells is linked to poor prognosis in PDAC. Perineural invasion is related to tumor cells' high levels of CD73+ expression. Significant correlations exist between high CD73 expression and PD-L1 expression in stroma and tumor cells. Patients who have lymph node metastases are more likely to have elevated CD73 expression in TILs [Tahkola K. et al, 2020].

In earlier investigations, comparable findings regarding CD73's effect on PDAC survival were presented. Stagg et al. found that CD73 loss enhanced the proportion of CD8+ T cells in tumors in a mouse study. This was considered to be one of the causes of CD73 deficiency's protective effects. An fatigued phenotype of T cells was linked to increased CD73 expression in T-lymphocytes in another animal model. It's possible that CD73 in PDAC affects TIL activity rather than TIL quantity to decrease immune response. In 2020, [Tahkola K. et al.]

Perineural invasion and high CD73 expression in tumor cells are related, suggesting that CD73 overexpression may play a role in this process. According to the research, when searched with thin slice thickness and also taking into account perineural invasion of low severity, perineural invasion can be discovered in some form in almost all surgically excised PDACs. The rate of perineural invasion was 71.7 percent, however, according to a meta-analysis of 3538 patients, which is consistent with other population studies [Tahkola K. et al., 2020]. This variation in occurrences between those discovered in normal histopathological analysis and those discovered in a thorough investigation using narrow slice thickness is assumed to represent the varying intensity of perineural invasion. In other words, when employing standard slice thickness, perineural invasion with mild severity may not always be detected in histological investigation. But according to the same meta-analysis, perineural invasion identified through standard histological investigation appears to be a standalone predictive factor for poor survival [Tahkola K. et al, 2020].

Significantly correlated with high PD-L1 expression in tumor cells is high CD73 expression in both tumor cells and stroma. Similar findings in gastrointestinal neuroendocrine tumors have been described. In a recent mouse study on head and neck cancer, Deng et al. showed a strong relationship between these two immunosuppressive chemicals. They demonstrated that downregulation of PD-1 and CTLA-4 on T cells caused CD73 blockage to revert

the fatigued T cell phenotype. Additionally, research on mice have demonstrated that inhibiting the adenosine receptor A2 (A2AR) improves the activity of anti-PD-1 antibodies by increasing antitumor T cell responses. In 2020, [Tahkola K. et al.]

Even while there is more and more proof that increased CD73 expression promotes tumor growth, the effects on TILs are still unclear. T helper 17 cells, myeloid-derived suppressor cells, and immunosuppressive regulatory T cells (T-reg) are reported to express CD73. We demonstrated that lymph node metastases were more common in PDAC patients with CD73 + TILs than in controls. The impact of the immunosuppressive cells indicated above may be reflected in this connection. To prove this theory, immune cells will need to be doubly stained in the future. Accordingly, Ma et al demonstrated that in head and neck squamous cell carcinoma, higher expression of A2AR was associated with a positive lymph node status. This is about the importance of the immunosuppressive adenosine pathway in the development of cancer. In 2020, [Tahkola K. et al.]

1. CD73 and gastrointestinal inflammation

Adenosine (Ado) and other nucleosides have an impact on almost every aspect of physiology and pathology. A group of ecto-nucleotidases, such as ectonucleoside triphosphate diphosphohydrolase-1 (CD39) and ecto-5'-nucleotidase (CD73), present on the surface of several cell types, metabolize extracellular nucleotides released at local regions of inflammation through controlled phosphohydrolysis. One of the four G protein-coupled Ado receptors can bind to and be activated by Ado once it has been produced. Ado is implicated in a wide range of tissue-protective mechanisms according to recent in vitro and in vivo research, which shed fresh light on the activities of adenosine. Ado receptors appear to couple to novel posttranslational protein modifications, such as Cullin deneddylation, as a new anti-inflammatory mechanism, according to studies in cultured cells and murine tissues. The Ado A2B receptor plays a crucial role in animal models of intestinal inflammation, according to studies on Ado receptor-null mice. Here, we discuss how Ado affects how cells and tissues respond to stress, with a focus on the gastrointestinal mucosa. [S.P. Colgan et al., 2013]

When Geoffrey Burnstock discovered that eATP and its derivatives affect gut and urinary tract neurotransmission in 1972, he introduced the purinergic signaling theory. When nucleotides bind to purinergic receptors, they modify cellular responses. They also act as mediators when ectonucleotidase hydrolyzes them into adenosine. After interacting with P1 adenosine receptors, adenosine controls cellular immunological responses [M. S. Longhi et al., 2019]

Ectonucleotidases are a group of enzymes that include ecto-nucleoside triphosphate diphosphohydrolases (ENTPDases), ecto-5'-nucleotidase (NT5E)/CD73, ecto-nucleotide pyrophosphate phosphodiesterases (E-NPPs), CD38/NADase, NAD glycohydrolases, ATP/ADP is hydrolyzed into AMP by the surface-located enzymes ENTPDase1, 2, 3, and 8. However, ENTPD2 has preferential ecto-ATPase activity. [M. S. Longhi et al., 2019]. While ENTPD1/CD39 and NT5E/CD73 are already present on a variety of immune cells, exposure to oxidative stress and hypoxia, activation with pro-inflammatory cytokines, or after engagement of the aryl hydrocarbon receptor (AhR) can further enhance their expression. [M. S. Longhi et al., 2019]. Inflammation of the gastrointestinal and hepatic systems has been linked to ENTPD1/CD39 and NT5E/CD73.

Both GPI-anchored protein and soluble enzyme have been used to define NT5E/CD73, the enzyme that turns AMP into adenosine. Both serum and cell-free lymph of healthy persons contain soluble CD73 (sCD73), which is mostly derived through lymphocyte shedding. In individuals with acute pancreatitis, an increase in sCD73 levels has been associated with inflammatory conditions and adversely connected with illness severity [Longhi M.S. et al., 2019].

In cases of intestinal and stomach disease, abnormal immune responses may lead to the emergence of persistent and escalating inflammatory conditions. In this context, numerous studies highlight the critical part that purinergic signaling plays in the control of GI conditions. [M. S. Longhi et al., 2019]

The chronic, crippling condition known as inflammatory bowel disease (IBD) is marked by increased colon and small intestine inflammation and is linked to thrombophilia and a higher risk of developing cancer. Evidence from both clinical and experimental studies suggests that ENTPD1/CD39 is protective in Crohn's disease (CD). In mice with dextran-sulfate-sodium (DSS)-induced colitis, a global ENTPD1/CD39 deletion increases vulnerability to damage. As a result, clinical remission in IBD patients who have high circulating T-reg ENTPD1/CD39 expression is correlated, whereas single nucleotide polymorphisms linked to low ENTPD1/CD39 mRNA levels enhance propensity to Crohn's disease [M. S. Longhi et al., 2019]

Reduced suppressor Th17-cells, a distinct effector cell subtype with immunosuppressive capabilities, are found in Crohn's disease patients. Compared to typical pathogenic Th17 cells, supTh17 cells express larger amounts of ENTPD1/CD39, produce eAMP and adenosine more efficiently, and can therefore powerfully inhibit effector T-cell responses via A2A receptors [M. S. Longhi et al., 2019]

IBD patients' lamina propria and peripheral blood show an increase in CD73+CD4+ T-cells, which are concentrated in lymphocytes that produce IL-17, during periods of active inflammation. In Crohn's disease, elevated CD73 levels have also been linked to the buildup of pathogenic Th17-cells. Unexpectedly, TNF exposure enhances CD73 expression on CD4+ T-cells whereas an anti-TNF monoclonal antibody (infliximab) has the opposite effects. This suggests that CD73+ Th17-cells may serve as a proxy for measuring disease activity and treatment response [M. S. Longhi et al., 2019]

A chronic inflammatory ailment called celiac disease is frequently linked to inflammatory bowel disease (IBD) and is brought on by erroneous immune reactions to dietary gluten. Recent studies have demonstrated that gluten exposure causes celiac patients' FOXP3+CD39+ T-regs to accumulate protectively. But these T-regs show poor suppression and are defective [M. S. Longhi et al., 2019].

There is proof that ENTPDase mRNA can be carried by microparticles (MPs) released by inflammatory site cells. Cells that incorporate into MPs may take up such mRNA material, which may then be translated into active NTPDases [M. S. Longhi et al., 2019]

2. CD73 and Gastrointestinal infections

The first point of contact between a host and a bacterium is the intestinal epithelium. IECs serve as the strongest physical barrier between the host and the intestinal microbiota because of their anatomical position between the intestinal lumen and the submucosal immune system. As intestinal barrier failure has been conclusively related to gastrointestinal illnesses including inflammatory bowel disease, this barrier is essential for the preservation of intestinal homeostasis. Tight junction development and mucin secretion contribute to the physical barrier, and released substances such antimicrobial peptides operate as a soluble barrier that directly affects luminal and surface-associated microorganisms to compartmentalize the intestinal microbiota. [D.J. Kominsky et al., 2017].

In the control of infection by the enteric pathogen S. Typhimurium, IEC CD73 has a new function. The single known activity of CD73, adenosine, had bacteriostatic effects on Salmonella growth start. [D. J. Kominsky et al., 2017]. This activity is quite specialized. In fact, none of the proximal upstream or downstream metabolites, excluding adenine, were as effective at inhibiting growth when present in equimolar quantities. It is also noteworthy that Salmonella's adenosine metabolism and the start of exponential growth occurred at the same

time. Salmonella either converts adenosine to inosine through the enzyme add or to adenine through the enzyme deoD. [D.J. Kominsky et al., 2017].

It's interesting to note that whereas Salmonella colonization appears to be suppressed by CD73 expression, Salmonella pathogenicity is affected in the opposite way. This shows that CD73 has a second crucial role in Salmonella infection, one that fosters the pathogen's virulence. As evidenced by the lowered weight loss and attenuated colon shortening in response to Salmonella colitis in mice, the severity of Salmonella colitis is attenuated in the absence of CD73 expression on the intestinal epithelium. After KD of CD73 expression, intracellular replication and bacterial translocation across the epithelium are hampered. For EPEC, Clostridium difficile, and Pseudomonas aeruginosa, the idea that extracellular adenosine or its downstream metabolites can increase the virulence of enteric pathogens has previously been put up; however, in each case, various pathways have been suggested. Our findings imply that Salmonella's intracellular localization is changed in the lack of CD73 expression, leading to poor intracellular translocation and replication. However, it is unknown what specifically caused this localization problem [Kominsky D.J. et al, 2017].

Adenosine's antibacterial activity was shown to be relevant using both an in vitro CD73 knockdown method and an in vivo conditional IEC knockout model. Salmonella colonization of the intestinal lumen after oral infection was significantly increased in the absence of IEC CD73 expression. While Salmonella invasion is unaffected, CD73 appears to have a major impact on intracellular localization, according to in vitro research employing IECs lacking CD73 expression. In particular, Salmonella's migration through cells from the apical to basal poles was slowed down and prevented by the absence of IEC CD73. Although the exact mechanism causing this slowed intracellular migration is unknown at this time, we believe that it hinders SCV development and localization to the juxtanuclear site. Salmonella bacteria may be less likely to lyse the vacuole in the absence of normal CD73 expression, and as a result, they may multiply more slowly than cytosolic bacteria. An growing role for adenosine as an innate immune mediator is supported by intraluminal adenosine activities, which are endogenous antibacterial in vivo. According to Kominsky D.J. et al. (2017), luminal adenosine is crucial for controlling the proliferation of luminal bacteria.

The anti-inflammatory function of CD73 in murine Salmonella infection was recently revealed by Alam et al. These researchers demonstrated that S. Typhimurium inhibits CD73 expression in a variety of organs, including lymphocytes and the gut. Proinflammatory cytokines were expressed at higher levels in CD73-knocked-out mice in response to S. Typhimurium infection. Similar to our findings, CD73 mice had less severe Salmonella infections, as evidenced by less weight loss after infection and less bacterial spread to the spleen and liver. These authors came to the conclusion that the increased inflammatory response that develops when CD73 expression is absent enables more effective pathogen clearance by innate and adaptive immune systems. Our use of an IEC conditional CD73 knockout rather than a whole-body knockout paradigm marks a key distinction between the current investigation and that of Alam et al. The absence of CD73 expression in the intestinal epithelium can be used to explain a large portion of the in vivo phenotype observed in both the current investigation and the study by Alam et al. As was seen in the study by Alam et al., dextran sulfate sodium salt-induced colitis in whole-body CD73 mice was previously reported to increase proinflammatory cytokine expression. It is unclear how the mechanism of decreased bacterial dispersion is influenced by this increased proinflammatory cytokine production. The intestinal epithelium is the site of the most important CD73-dependent host-pathogen interactions, and in both IEC-specific and whole-body CD73 mice, the course of infection is largely determined by these interactions. This is in line with the finding that colonization disparities exhibited in whole-body KO mice are mimicked by conditional KO mice's increased colonization of the liver and spleen. The intestinal epithelium's lack of CD73 significantly lowers bacterial translocation, which in turn reduces systemic dispersion and, thus, reduces colonization of distant sites. Therefore, by pinpointing the intestinal epithelium as the critical region of CD73 expression that governs the course of local and systemic infection, our findings greatly expand our understanding of the role of CD73 in Salmonella infection [Kominsky D.J. et al, 2017].

Over 50% of people have a lifetime infection due to Helicobacter pylori. Infection causes chronic antral gastritis in both adults and children, which is defined by the infiltration of polymorphonuclear and mononuclear leukocytes into the mucosa. Additionally, this infection results in gastroduodenal ulcer, gastric lymphoma, and gastric cancer. [M.S. Alam et al., 2009]

Gastritis that is chronically active is brought on by H. pylori or Helicobacter felis infection in C57BL/6 mice. According to a number of studies, the response to stomach infection involves a selective increase in T helper (Th) type 1 (Th1) cells. Other research has shown that various CD4+ T lymphocyte subpopulations have unique functions in mediating and controlling H. pylori-induced gastritis. For instance, cotransfer of CD4+CD45RBlo regulatory T cells, sometimes known as "T-reg," protects against gastritis when adoptive transfer of CD4+CD45RBhi effector T cells from naive donors to immunodeficient recipients causes severe gastritis in recipients infected with H. pylori. Other studies have shown that T-reg are crucial in reducing immunological reactions to infections and natural flora. [M.S. Alam et al., 2009]

Adenosine is one mediator that has been linked to the regulation of the host's response to infection. Purine nucleoside adenosine builds up in inflammatory or hypoxic tissues primarily as a result of CD39 nucleoside triphosphate dephosphorylase, which mediates the dephosphorylation of ATP to ADP and then to 5'-AMP and CD73 (ecto-5'-nucleotidase), catalyzing the terminal reaction to convert 5'-AMP to adenosine. Four G-protein-coupled receptors mediate the many adenosine-controlled responses (A1, A2A, A2B, and A3). T cell A2A adenosine receptor (A2AAR) activation results in a number of reactions that are classified as anti-inflammatory. Our team has previously stated that the presence of A2AAR is necessary for T-reg-mediated regulation of colonic inflammation. Subsequently, further investigations have demonstrated that T-reg express CD73 and that its presence promotes T-reg activity through the synthesis of adenosine. Little is currently understood about the function of the T-reg or how adenosine affects the host's reaction to Helicobacter species infection. [M.S. Alam et al., 2009]

According to a recent study by Deaglio et al., in murine Th cells, the expression of CD73 by T-reg and the presence of the A2AAR receptor on activated effector Th cells lead to immunosuppressive loops where T-reg produce adenosine, which limits the activity of effector Th cells. Similar to this, Kobie et al. demonstrated that mouse T-reg produce CD73, which changes extracellular sources of 5'-AMP into adenosine, which in turn inhibits effector Th cells. CD73 is broadly expressed and highly inducible with activation. The capacity of T-reg to produce adenosine from ATP and ADP can considerably aid in their capacity to inhibit effector cells during infection-induced inflammation since CD73 are rate-limiting for extracellular adenosine production. Furthermore, effector Th cells or other cells may add to the adenosine pool that helps to limit host reactivity by expressing CD73 on its own [M.S. Alam et al., 2009]

T-helper cells play a crucial role in antibacterial reactions. However, prolonged release of Th1-related cytokines, like in the case of Helicobacter (H.) pylori infection, contributes to chronic inflammation that may eventually lead to peptic ulcer disease and stomach cancer. Insufficient Th1 immunity, however, can result in persistent infection because regulatory T-cell (T-reg) accumulation promotes pathogen persistence. In vitro and in animal models of H. felis-induced gastritis, adenosine production by ENTPD1/CD39 and CD73 on T-reg and memory T-cells reduces effector T-cell immunity substantially [Longhi M.S. et al, 2019].

The gastritis in CD73 mice is more severe and is accompanied by elevated levels of pro-inflammatory cytokines and compromised T-reg activity. By reducing levels of TNF- and IFN-, the administration of an A2A adenosine receptor (A2AR) agonist to Il-10 and Helicobacter-bearing mice reduces gastritis [M. S. Longhi et al., 2019]

In mouse models of intestinal (and systemic) Toxoplasma gondii (T. gondii) infection, a similar regulatory pathway has been found. Traditional CD4+ T-cells and T-regs both express ENTPD1/CD39 and CD73 in the gut of naive mice. Adenosine production is decreased as a result of the downregulation of CD73 expression during acute T.

gondii infection. Administration of receptor agonists reduces illness symptoms and the resulting dysbiosis as long as levels of type-1 purinergic adenosine receptors are maintained [M. S. Longhi et al., 2019]

3. CD73 and Gastrointestinal Cancers

Neuroendocrine tumor (NET) G1, NET G2, neuroendocrine carcinoma (NEC), and mixed adenoendocrine carcinoma (MANEC) groups are the subgroups of gastrointestinal neuroendocrine neoplasms (GI NENs) according to the WHO 2010 classification. The WHO 2010 classification was used to evaluate 136 cases of GI NENs that were identified at our hospitals as gastrointestinal carcinoids, endocrine cell carcinomas, and NENs throughout the course of the previous 11 years. The NET group (NET G1/G2) comprised 88.2% (120/136) of the 136 cases, whereas the NEC group (NEC/MANEC) comprised 11.82% (16/136) of the cases. When compared to the NET group, the NEC group had greater occurrences of lymphatic and venous invasions (P=0.0001 and P=0.0021, respectively). In GI NENs, the immunohistochemistry staining of CD73 (cluster of differentiation 73) was assessed. In terms of tumor immunity, CD73 may be helpful [Takimoto M. et al., 2017].

Adenosine monophosphate is often converted to adenosine by CD73 on the tumor cell membrane, which inhibits interferon production and cytocidal action. Few studies have examined CD73 expression in GI NENs, despite reports of a link between CD73 and stem cells from pancreatic NENs. In 27.2 percent (37/136) of the GI NENs, immunohistochemical CD73 expression on the cytomembrane of neuroendocrine cells was found. When compared to the NET group, the NEC group's positive CD73 ratio was noticeably greater (P=0.0015). [M. Takayoshi et al., 2017] . A possible biomarker for anti-programmed death 1 (PD 1) therapy is CD73. On the cytomembrane of GI NENs, the expression of programmed death ligand 1 (PD L1) was evaluated. In comparison to the NET group, the NEC group had a greater positive ratio of PD L1 (P=0.0011). Additionally, there was a strong correlation between the expression of PD L1 and CD73 (P 0.0001). These findings suggest that CD73 might be a promising biomarker for some prognostic and therapeutic variables related to PD 1 therapy [Takimoto M. et al, 2017].

Langhans first identified neuroendocrine tumors (NENs) in 1867. Oberndorfer coined the term "carcinoid" (or "karzinoide") for the tumors in 1907. They had been regarded as benign tumors. These tumors are now understood to be cancerous, nevertheless. The majority of the body's organs develop NENs. Typically, they are concentrated tumors of a single organ system, like the lungs, pancreas, or digestive system. On microscopic examination, NENs are made up of ovoid or spherical cells with granular cytoplasm and "salt and pepper"-appearing nuclei. The cells frequently form nests, tiny follicles, or structures resembling glands. [M. Takayoshi et al., 2017]

Neuron-specific enolase, synaptophysin, chromogranin A (CGA), cluster of differentiation 56 (CD56), and other immunohistochemistry neuroendocrine markers are used to identify NENs (NSE). The most specific neuroendocrine marker is CGA, while synaptophysin is thought to be the most sensitive sign. Therefore, it is advised to use both synaptophysin and CGA in the usual pathological diagnosis of NENs. Few studies have examined the relationship between immunohistochemical CGA, synaptophysin expression, treatments, and prognosis. [M. Takayoshi et al., 2017]

Gastrointestinal NENs (GI-NENs) are not classified uniformly in Japan when they are diagnosed. Numerous pathologists in Japan make GI-NEN diagnoses based on the original classification for each organ. For instance, the Japanese Classification of Colorectal Carcinoma was used to detect a colorectal neuroendocrine tumor (NET) (JCCC). According to the WHO 2010 classification, numerous pathologists have been diagnosing GI-NENs in recent years [Takimoto M. et al, 2017].

Cancer immunotherapy has advanced significantly in recent years as a result of improvements in our understanding of tumor biology and immunology. Extracellular adenosine monophosphate (AMP) is an important molecule that is enzymatically dephosphorylated and converted into adenosine and inorganic phosphate by a crucial enzyme known as cluster of differentiation 73 (CD73), also known as ecto-5’-nucleotidase.

Through the adenosine A2A receptor (A2AAR) on T cells and natural killer cells, adenosine inhibits the synthesis of INF- and cytocidal activity. Antitumor immunity is boosted in animals lacking functional A2AAR and CD73, which slows the formation of tumors. Recent research has shown that CD73 is both a novel molecular target for pancreatic NENs (pNENs) therapy and a distinct biomarker for pancreatic NENs (pNENs) cancer stem cells. The cytomembrane of GI-NENs with a high malignant potential may express CD73. A promising biomarker for anti-programmed death-1 (PD-1) therapy is CD73, according to Takimoto M. et al. (2017).

Immunohistological CD73 expression is related with tumor growth in A2AAR-associated tumors rather than the diagnosis of GI-NENs like CGA or synaptophysin. Recent research has identified CD73 as a novel molecular target for pNENs therapy as well as a distinctive biomarker for pNENs cancer stem cells. Numerous tumor forms, including colorectal cancer, gastric cancer, gallbladder cancer, serous ovarian cancer, triple negative breast cancer, and malignant melanoma, exhibit CD73 expression, which is likewise linked to a bad prognosis.] Takimoto M. et al.

With enzyme assays in tissue homogenates or tissue slices, there were no changes in CD73 expression between endometrial cancers. As far as we are aware, there hasn't been any information about CD73 expression in GI-NENs. Additionally, synaptophysin, CGA, and CD73 do not appear to be significantly correlated. The membrane of GI-NENs with a high malignant potential expresses CD73. In the current investigation, we also used immunohistochemical labeling to look at CD73 expression on the cytomembrane of GI-NENs. In comparison to the NET group, the immunohistochemistry CD73 expression ratio was greater in the NEC group. In the current study [Takimoto M. et al., 2017], greater CD73expression is also linked to an increased malignant potential of GI-NENs.

Lymphatic and venous invasions have been found to be distinct predictors of lymph node metastases in colorectal NENs. In addition, instances with tumors smaller than 10 mm in size and lymphatic and venous invasions are more likely to develop colorectal NENs that metastasize to the lymph nodes. Additionally, it has been noted that in pNENs, invasion into neighboring organs was substantially linked with the immunohistochemistry expression status of CD73. In the current study, the NEC group experienced higher lymphatic and venous invasions than the NET group. In our investigation, there was no correlation between lymphatic and venous invasions and CD73 expression determined by immunohistochemistry. According to Takimoto M. et al. (2017), it is assumed that lymphatic and venous invasions are not connected to the CD73-related pathway.

One potential biomarker for PD-1 treatment is CD73. Targeted inhibition of CD73 may amplify therapeutic approaches that target immune checkpoint inhibitors more broadly by enhancing the therapeutic activity of anti-PD-1 and anti-CTLA-4 monoclonal antibodies. A key factor in controlling adaptive immune responses and preventing autoimmune and auto-inflammatory reactions in a healthy host is the checkpoint molecule PD-1, which is present on T cells. Variable levels of PD-L1, its main ligand, are expressed on tumor-associated antigen-presenting cells and cancer cells. It is unmistakably linked to a favorable outcome for therapy with PD-1/PD-L1 blocking antibodies, which can slow the growth of tumors. The rabbit monoclonal antibody 28-8 may uniquely identify the PD-L1 plasma membrane protein expressed in cancer cells in PD-L1 immunohistochemistry assays. According to Takimoto M. et al. (2017), there is a significant link between the expressions of CD73 and PD-L1 on the cytomembrane of GI-NENs.

In gastric adenocarcinoma (GAC), the biological function of CD73 and its translational importance are mostly unknown [Dominici M. et al, 2021].

One study found that the CD73 gene was among the top ten genes related with GAC survival, and that it had the greatest effect on survival among clinically verified genes with this condition when combined with FGF1. In terms of its effect on survival, the overall survival (OS) of GAC patients with high CD73 expression was lower than that of the low CD73 group. To determine the clinical importance of CD73's value in GAC, the expression pattern, co-expression networking, and prognostic significance in GAC have been evaluated. [M. Domino et al., 2021]

The discovery that adenosine signaling, in particular adenosine A2A receptor (A2AR) signaling, is a potent suppressor of tissue-devastating immune cell responses, as well as studies focusing on CD73 in breast cancer, melanoma, and non-small cell lung cancer, have all paved the way for these findings over many years of research. One of the main causes of cancer-related deaths is gastrointestinal (GI) cancer. Immunomodulatory approaches, either alone or in conjunction with existing therapies, are increasingly being shown to have promise for improving patient outcomes. 2020 Harvey J.B. Recently, the FDA approved the use of numerous immune checkpoint inhibitors in GI malignancies; nevertheless, the therapeutic effect is modest. Examining immunosuppressive molecular processes in GI malignancies, such as CD73, can support current initiatives to increase the number of patients who benefit from immunotherapy. With a particular focus on CD73's potential as an immunotherapy target in these cancers, we describe current clinical and fundamental research findings on CD73 in GI cancers, including gastric, liver, pancreatic, and colorectal cancer, in this review. 2020 [Harvey J.B.]

The majority of cancer-related deaths globally are caused by gastrointestinal (GI) cancers, which are among the most prevalent cancers worldwide. Antibodies against programmed death-1 (PD-1) and immune checkpoint inhibitors (ICIs) have been approved for use in gastrointestinal malignancies. Even though their approval represented a huge step forward in clinical care, only a small number of patients currently benefit. The majority of patients who benefit have malignancies with low levels of DNA mismatch repair (dMMR) and high levels of microsatellite instability (MSI-H). 2020 [Harvey J.B.]

In contrast, competent MMR tumors, which make up the great majority of GI cancer patients, have not responded favorably to ICIs when used alone. MSI-H tumors are the cause of 6–22% of gastric, 1% of pancreatic, 3% of liver, and 14–16% of colorectal cancers. The most sophisticated immunotherapy for cancer uses antibodies against PD-1/programmed death-ligand 1 (PD-L1) or cytotoxic T lymphocyte-associated protein-4 (CTLA-4). The PD-1/PD-L1 axis stimulates the development of regulatory T cells while suppressing effector T cells to increase adaptive immunological resistance (T-regs). Additionally, CTLA-4 functions as a negative regulator of T cells, preventing T cell activation by binding to B7-1 or B7-2 on antigen-presenting cells. Combination ICI therapy is progressing in preclinical and clinical settings, as are new strategies to harness the immune system, such as vaccines and viral therapy, adoptive cell transfer, and cytokine therapy, to increase the efficacy of immunotherapy for more patients. 2020 [Harvey J.B.]

The ability to stimulate de novo immunogenicity and the ability to overcome the tumor microenvironment's immunosuppressive activities are among the difficulties with increasing efficacy. As a promising target for reestablishing anticancer immunity, CD73 antibodies and small molecule inhibitors have recently entered clinical trials. The current research on CD73 in GI malignancies and its potential as an immunotherapy target is summarized in this review. The clinical implications for GI cancers are being studied in ongoing clinical trials that combine ICI and conventional therapy with CD73 and adenosine receptor targeting. 2020 [Harvey J.B.]

The third most prevalent cancer worldwide and the fifth most common cancer worldwide, respectively, is gastric cancer (GC). Advancements in prevention and treatment continue to be a priority, despite the fact that incidence and death rates are dropping. Once the cancer has spread past the stomach lining, the five-year survival rates fall to 20-30% or less. Most GC situations are in an advanced state. Gastric resection, radiation, chemotherapy, and targeted therapy, such as antibodies against (VEGF)/VEGF receptor 2 (VEGFR2) and HER2, are all part of the treatment. Recently, ICI therapy for GC received approval. But the majority of patients do not gain. The combination of ICI therapy, adoptive cell transfer, vaccinations (such as melanoma-associated antigen (MAGE) A3 peptides and Bacillus Calmette-Guerin (BCG)), and agonist antibodies for costimulatory receptors are additional immunotherapies being researched in GC [Harvey J.B., 2020].

The expression of CD73 in GC has not been extensively studied. When compared to normal tissue, GC exhibits higher levels of CD73 expression, which is linked to advanced disease, metastasis, poor overall survival, increased depth of invasion, and positive nodal status. Hypoxia might contribute to an increase in CD73 in GC. In gastric cancers, hypoxia-inducible factor-1 (HIF-1) staining and high CD73 expression are tightly correlated. High CD73 expression, on the other hand, is associated with good overall survival in GC, according to gene expression

studies. The discrepancies between these findings may be explained by the fact that CD73 expression does not necessarily correlate to protein expression. Furthermore, GC has considerable CD73 heterogeneity. For instance, low or no expression of CD73 is found in 30–50% of advanced stage, highly aggressive, and lymph node positive cancers [Harvey J.B., 2020].

10–20 percent of colorectal cancers have BRAF mutations, which are usually MSI-H (. NT5E promoter methylation, which has been shown in both breast and melanoma cancer, has an effect on CD73 expression as well. It should go without saying that a variety of molecular and genetic variables can influence CD73 expression in human malignancies [Harvey J.B., 2020]. In the future, studying CD73 expression in relation to frequent molecular and/or genetic abnormalities in GC and The Cancer Genome Atlas (TCGA) may aid in understanding CD73 in GC. Studies examining the relationship between immunological checkpoints like PD-L1 and CD73 expression may also be useful. Preclinical studies indicate that high CD73 expression in PD-1/PD-L1 expressing tumors may identify individuals who might benefit from combination anti-PD-1/PD-L1 therapy and CD73 and/or A2AR inhibition. Forty percent of GC cases are PD-L1 positive. Few studies have examined the relationship between CD73 expression and other ecto-enzymes involved in ATP and adenosine synthesis, metabolism, and its intracellular uptake, including other E-NTPDases, ecto-nucleotide pyrophosphatases/phosphodiesterases (e.g., CD203a), nitcotinamide dinucleotide enzymes (e.g., CD38), prostatic acid phosphat (ENTs and CNTs, respectively). This may provide a significant obstacle to creating efficient adenosine-based medicines, according to a recent review by Boison and Yegutkin. In light of this, a broader perspective on the metabolism and signaling of extracellular adenosine in GC may also be important [Harvey J.B., 2020].

Studies of CD73's connection to H. pylori-mediated carcinogenesis may offer further explanation in light of CD73/extracellular adenosine's function in immune cell escape. Up to 60% of GC cases are caused by an infection with H. pylori that develops in the context of inflammation. Evidence that PD-L1 expression is higher in H. pylori positive gastric biopsies compared to negative gastric biopsies and that H. pylori-induced PD-L1 expression on gastric epithelial cells converts naive T cells to CD4+ FoxP3+ T-regs that inhibit T cell proliferation support the idea that immune cell evasion is important for H. pylori infection. By boosting local extracellular adenosine, which inhibits IFN- production, CD73 expression by CD4+ CD25+ T-regs promotes H. pylori infection. In line with this, infected CD73-deficient animals develop more severe gastritis and inflammation (such as elevated IL-2, TNF-, and IFN-, as well as compromised T-reg activity). Together, these results provide evidence that CD73/extracellular adenosine may work with other immunological checkpoints to inhibit the immune system's ability to recognize and eliminate altered cells that arise in persistently infected gastric tissues, hence promoting the growth of GC. Exosomes containing CagA and VacA are produced from gastric epithelial cells as a result of H. pylori infection, inducing pro-inflammatory reactions and altering the expression of tumor suppressor and oncogenic genes. It would be interesting to find out if CD73 is expressed on H. pylori-mediated exosomes and if its presence or increased presence is a biomarker for the development of GC given that CD73 expression on exosomes increases tumor immunosuppression. 2020 [Harvey J.B.]

Additional research demonstrates that CD73 encourages GC stemness, migration, proliferation, and invasion of tumor cells. Extracellular adenosine has been implicated in several antitumor processes, including AMP-kinase (AMPK)-mediated, caspase-independent apoptosis, which is mediated by ENTs, and caspase-dependent apoptosis, which is mediated by A1R and A3R. 2020 [Harvey J.B.]

Liver cancer ranks sixth globally in terms of incidence and is the fourth most prevalent cause of cancer death. Hepatocellular carcinoma makes up 90% of liver cancers (HCC). Hepatitis B or C infection or chronic alcohol consumption are the most common causes of chronic liver disease, which includes cirrhosis and fibrosis. 18% of HCC patients survive the disease after five years. Targeted therapy, liver transplantation, and tumor removal are all included in treatment. However, due to advanced disease, 70% of patients are ineligible for surgery. Recently, ICI therapy was approved as a second-line treatment for HCC. In addition to combination ICI therapy, oncolytic viruses, vaccines, and other promising immunotherapies are being developed with the goal of enhancing preexisting or new immune responses [Harvey J.B., 2020].

In recent years, CD73's new biology in human cancers has been uncovered thanks in part to HCC. Snider and colleagues' research revealed that the NT5E-2 alternative splicing variant is expressed in liver cirrhosis and HCC. Because exon 7 is lost during splicing, NT5E-2 generates the human-specific protein CD73-short (CD73s), which lacks enzymatic activity and cannot dimerize. The cytoplasm is the only place where CD73 is expressed, and it interacts with CD73 to facilitate the proteasome-dependent degradation of CD73. While CD73 expression is downregulated by more than 90% in HCC human tissues, CD73s expression is 6–8 times higher than in healthy liver. According to certain research, HCC patients with CD73 overexpression have poor tumor differentiation, microvascular invasion, and overall and recurrence-free survival. 2020 Harvey J.B. Sciarra et al. specifically reported substantial cytoplasmic CD73 expression in malignancies, particularly with invasive cancers, in accordance with CD73s expression.

Numerous immunohistochemical results for malignancies with strong CD73 expression, such as pancreatic and gastric cancer, demonstrate considerable CD73 cytoplasmic staining. Alcedo et al. find that abnormal glycosylation severely restricts CD73 enzyme activity in HCC. Contrary to healthy hepatocytes, CD73 in HCC cells has aberrant N-linked glycosylation in its C-terminal catalytic domain, which significantly reduces CD73's ability to function as an enzyme. These results further highlight the possibility that CD73 protein expression levels might not always correspond to the protein's capacity to produce extracellular adenosine. 2020 Harvey J.B.

Therefore, research that combines the tissue- and cell-specific functions of CD73 in healthy GI tissues may aid in our understanding of CD73 in GI malignancies. CD73 is downregulated in cancer cells of bladder and prostate tumors, which is associated with a poor prognosis and is similar to endometrial cancer. It is unknown what function CD73 serves in the bladder and prostate epithelium. Studies by Vecchio et al. in prostate cancer describe a constitutively active, ligand-independent A2BR that promotes the growth of cancer cells [Harvey J.B., 2020].

Studies on adenosine receptors reveal that A2AR signaling through PI3K-AKT enhances the growth and spread of HCC tumors and is inhibited by the use of A2AR antagonists. In human HCC tissue, A2BR expression is elevated, corresponds with tumor growth, and is probably caused by hypoxia. 2020 [Harvey J.B.]

By 2030, pancreatic cancer is expected to overtake lung cancer as the second biggest cause of cancer-related fatalities in the US. Pancreatic ductal adenocarcinoma (PDAC) makes up 90% of pancreatic cancers, while neuroendocrine tumors make up 3-5%. (PNETs). Risk factors include smoking, binge drinking, being overweight, having H. pylori infection, and having chronic pancreatitis. The prognosis is exceedingly bad; almost 70% of patients will pass away from the condition within the first year. Nine percent of people survive for five years. Radiation treatment, chemotherapy, and targeted therapy are all part of the standard of care for pancreatic cancer (e.g., EGFR inhibitors). A recurring issue is the occurrence of therapeutic resistance to various medicines. Despite increased expression of PD-L1 in tumors, single agent or combination ICI therapy for PDAC patients has not been effective. 2020 [Harvey J.B.]

When compared to healthy pancreatic tissue, PDAC exhibits an upregulation of CD73, which is associated with a worse prognosis, a larger tumor, an advanced stage, lymph node involvement, and metastasis. Even though PDAC tumors exhibit 100% positivity for CD73 expression, CD73 staining patterns are visible. PDAC cells with good to moderate differentiation have mixed membrane and cytoplasmic CD73 staining. These tumors exhibit low to moderate CD73 staining intensity. The CD73 labeling is abnormal in PDAC cells with poor differentiation, and CD73 expression is highly expressed in the cytoplasm. HCC has been found to include CD73s. Studies examining the expression of NT5E-2 (CD73) could provide more insight into CD73 in PDAC. When compared to wild-type tumors, CD73 staining is more pronounced in human colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) tissue [Harvey J.B., 2020]. In CRC and NSCLC cell lines, KRAS changes are associated with elevated CD73, A2AR, and A2BR gene expression, which is correlated with anti-PD-1 resistance in KRAS mutant tumor models. In addition, compared to patients with KRAS mutations and tumors with low CD73 expression, patients with high CD73 expression and KRAS abnormalities have lower overall survival. A low overall survival rate is likewise associated with EGFR mutations and high CD73 expression. In 67 percent of PDAC cases, KRAS mutations and

EGFR changes coexist. As a result, PDAC may also express more CD73 as a result of EGFR changes. In breast cancer, CD73 expression and EGFR changes are shown to be positively correlated. 2020 [Harvey J.B.]

In PDAC, CD73 expression rises with more severe disease, which could be a sign of a developing or advanced immunosuppressive phenotype. For instance, as the disease progresses in PDAC, there is a decline in CD8+ T cell infiltration into tumors and an increase in infiltrating T-regs. Human T-regs hardly ever express cell surface CD73, and it is thought that extracellular adenosine produced by CD73 from other sources activates adenosine receptors on immune cells to decrease immunity. In light of this, extracellular adenosine-mediated immunosuppression may be significantly supported by the concurrent upregulation of CD73 in PDAC cells. Furthermore, tumor-infiltrating CD11b+ CD103- DCs stimulate the proliferation of CD73+ tumor-promoting T-regs in PDAC model tumors [Harvey J.B., 2020].

In PDAC cells, CD73 has also been shown to encourage treatment resistance and tumor formation. The tumor's interaction with these cells and the milieu around them are thought to result in various reactions and results. For a better understanding of the potential therapeutic benefit of targeting CD73 and adenosine receptors in pancreatic tumors, in-depth and detailed characterization of CD73/extracellular adenosine in immunocompetent, autochthonous pancreatic cancer models, humanized models, and human organoids will be crucial in the upcoming years. 2020 Harvey J.B.

Incidence rates for colorectal cancer (CRC) are decreasing in the United States and stable in the majority of other Western nations, while they are increasing in Eastern Asia and Eastern Europe, largely as a result of a Westernized lifestyle. However, it is the second cause of cancer-related deaths globally and the third most prevalent cancer. Obesity, a Western diet, a lack of exercise, binge drinking, genetic disorders, and smoking are all CRC risk factors [Harvey J.B., 2020]. Surgery, combined chemotherapy, radiation therapy, and targeted therapy, such as antibodies against VEGF/VEGFR or EGFR, are all forms of treatment. Although improvements in screening and treatment have been made over the past ten years, people with metastatic CRC still have a dismal prognosis. 15% of people survive for five years. Only 4% of dMMR/MSI-H CRC cases have metastatic disease. In order to increase the efficacy of immunotherapy for more patients, a number of strategies are being researched, including IDO inhibitors, vaccine therapy, and combined ICI therapy. These attempts might be aided by a deeper comprehension of CD73/adenosine receptor signaling in CRC [Harvey J.B., 2020].

Early research on CD73 in CRC was done as part of wider studies looking at the enzymatic patterns of important enzymes involved in purine metabolism and salvage, such as ADA, alkaline phosphatase, and hypoxanthine-guanine phosphoribosyltransferase. There was no difference in CD73 enzyme activity between CRC and normal tissue, according to research by Camici et al. Eroglu et al. found that tumors exhibit higher CD73 enzyme activity than normal tissue. Poor clinical characteristics and high CD73 enzyme activity did not show any correlations. Instead, it was discovered that well-differentiated tumors had high CD73 enzyme activity, whereas tumors with low CD73 enzyme activity had low CD73 enzyme activity [Harvey J.B., 2020]. Recent investigations have demonstrated a correlation between high CD73 expression and poor tumor differentiation, lymph node involvement, advanced stage, and poor survival. Different cell types that express CD73 have different clinical prognoses for rectal cancer. Similar to bladder cancer. Epithelial cell expression of CD73 is associated with higher overall survival and progression-free survival, whereas stromal cell expression of CD73 is associated with less favorable outcomes. These results confirm that CD73 in tumors may promote and prevent tumor growth. Targeting cancers that have two distinct functions for CD73 may be difficult with CD73 inhibitor therapy, while this is uncertain [Harvey J.B., 2020].

Through -catenin (WNT)/cyclin D1 signaling, CD73 encourages the formation of CRC tumors and CRC cell proliferation. It's likely that KRAS mutations or changes also affect CD73 expression in CRC. Recent investigations have revealed that low survival and high CD73 expression are associated with a noticeably shorter time to recurrence. An adenosine high expression profile was found in pretreatment biopsies of renal cancer patients

and was connected to the clinical response to A2AR antagonism. Similar efforts to uncover biomarker signatures could considerably enhance the effectiveness of immunotherapy for CRC. While many adenosine pathway members collectively suggest potential therapeutic targeting in CRC, more thorough research in human tumors and pertinent preclinical models are urgently required. 2020 [Harvey J.B.]

The second most common cause of tumor-related death in the US is colorectal cancer (CRC). The two main ectonucleotidases expressed by tumor endothelial cells and T-regs are ENTPD1/CD39 and CD73. [M. S. Longhi et al., 2019]

Furthermore, there is proof that CD8+ lymphocytes infiltrating human CRC can identify a variety of epitopes that are unrelated to the tumor, such as those that were present during earlier viral infections. According to the patients' clinical status, these CD8+ cells exhibit a wide range of ENTPD1/CD39 expression [Longhi M.S. et al., 2019].

Ren et al. assessed the immunohistochemistry expression of CD73 in oral squamous cell carcinoma (OSCC), demonstrated the relationship between this marker and the clinicopathological features of such patients, and hypothesized that CD73 might be a predictive diagnostic for OSCC. Regarding the prognostic value of CD73 expression in tumor cells in patients with salivary gland malignancies, there aren't any data yet [Ranjbar M-A. et al., 2019]. Approximately 5% of head and neck malignancies are salivary gland neoplasms. Such unusual illnesses are frequently difficult to diagnose. For oral pathologists and surgeons, the overlapping characteristics of different forms of salivary gland cancers may make diagnosis difficult. Pleomorphic adenomas are the most frequent benign tumors of the salivary glands, while mucoepidermoid carcinoma and adenoid cystic carcinoma are the most frequent malignant tumors. It might be challenging to assess salivary gland neoplasms utilizing histological examination by hematoxylin-eosin (H&E) staining. Therefore, for a certain diagnosis, it is recommended to use immunohistochemistry (IHC) in addition to H&E staining to distinguish between these related malignancies [Ranjbar M-A. et al., 2019].

IHC is an essential but limited diagnostic tool for salivary gland malignancies, according to the most recent research. It should be used after a thorough H&E staining evaluation for cancer. IHC can be used to support and facilitate the histiopathological examination for a certain diagnosis. [M-A. Ranjbar et al., 2019]

According to the information currently available on CD73 immunoreactivity in other tumors, high levels of CD73 expression are associated with a poor prognosis for colorectal cancers. Additionally, CD73's biological characteristics have been shown to be useful for identifying patients with developing tumors. Additionally, another study examined CD73 expression in patients with gallbladder adenocarcinoma and found that CD73 overexpression was related to tumor growth and patient survival. It is known that CD73 is a separate marker for the clinical characteristics and prognosis of gallbladder cancer [M-A. Ranjbar et al., 2019]

To prove that CD73 is a prognostic and diagnostic marker in salivary gland cancers, more molecular research are required [Ranjbar M-A. et al., 2019].

2. ROLE of CD73 IN HEPATOLOGY

1. CD73 FUNCTIONS IN THE LIVER

Although at lower levels than in hepatocytes, CD73 is expressed on the apical membrane of endothelial cells and hepatocytes in the normal liver. When stimulated hepatic stellate cells are differentiated into myofibroblasts in vitro, NT5E is increased. Additionally, in response to hepatic inflammation, regulatory B cells that are CD73+ are

attracted to the liver. According to Minor M. et al. (2019), CD73 has become an important regulator of hepatocyte responses to various types of injury, shedding light on common disease mechanisms that may be used therapeutically. Although the specific adenosine receptor type that caused these effects could not be identified, a prior study suggested that A2ARs may protect the liver from hepatic I/R injury by suppressing natural killer cells. Another option is that the A2BR, which has been implicated in cardiac protection by IP as shown by pharmacological and genetic techniques, may mediate the protective effects of CD73 in hepatic protection by IP [Minor M. et al, 2019]. In a mixed cohort of male and female mice, genetic deletion or pharmacological suppression of the A2AR enhanced inflammation and liver damage after concanavalin A liver injury [Minor M. et al, 2019]. By producing adenosine from AMP breakdown, the tumor-expressed CD73 ectonucleotidase induces immunological tolerance and encourages invasiveness. Although anti-CD73 blockade therapy is a promising method for cancer immunotherapy, the human hepatobiliopancreatic system's CD73 expression has not been sufficiently characterized. In a variety of non-neoplastic and neoplastic diseases of the liver, pancreas, and biliary system, immunohistochemistry was used to examine CD73 expression [Sciarra A. et al, 2019].

Adenosine may play a profibrogenic role, and CD73 has also been linked to the development of liver fibrosis. It is unknown how liver CD73 expression, distribution, and enzymatic activity are impacted by the formation of hepatic fibrosis. An additional study found that CD73 was expressed near fibrotic nodules, but it did not measure the levels of protein expression or the distribution of different cell types. In the context of liver fibrosis, the cell type-specific roles of CD73 in hepatocytes as opposed to other cell types, such as myofibroblasts, immune cells, or endothelial cells, have not been investigated in a quantitative and dynamic way. This kind of knowledge will be essential to comprehending extracellular adenosine's role in liver fibrosis and may clarify some of the conflicting findings that have been reported on the anti- and profibrotic properties of adenosine and its receptors. In 2019, Minor M. et al.

In both mice and people, the development of liver disease and hepatocellular damage is strongly influenced by genetic predisposition. CD73 was found to be a key participant in hepatocyte injury, which is characterized by ballooning degeneration and the production of cytoplasmic protein aggregates in liver injury-susceptible and -resistant mice strains. Mallory-Denk bodies (MDBs), which contain cytoskeletal components and stress-activated proteins, are frequently found in the hepatocytes of people with chronic liver disease. Through repeated administration of the medication 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC), Nt5e mice are shielded against the hepatocellular damage caused by MDB [Minor M. et al., 2019]. The Nt5e mice exhibit diminished hepatomegaly and no detectable MDBs upon histological analysis, despite the fact that they continue to experience liver injury (as seen by higher serum liver enzyme levels). Given that WT animals exposed to this injury paradigm have drastically downregulated cell-surface CD73 enzymatic activity and protein expression, this response is somewhat perplexing. The Nt5e model suggests that CD73 promotes MDB, yet CD73 accumulates intracellularly in hepatocytes exposed to DDC, raising the hypothesis that CD73's effects on MDB are not always related to its role as an ecto-AMPase [Minor M. et al, 2019]. Human hepatocellular carcinoma (HCC), the most prevalent kind of primary liver cancer, exhibits MDBs. Intriguingly, tumor and surrounding non-tumor tissue in human HCC exhibit cytoplasmic increase and loss of plasma membrane-associated CD73 [Minor M. et al, 2019].

In healthy hepatobiliopancreatic tissues, CD73 is expressed. In contrast, it was only found in a small subset of pancreatic neuroendocrine neoplasms and was almost completely absent in acinar cell carcinoma. It is present in all hepatocellular carcinoma (HCC), all pancreatic ductal adenocarcinoma (PDAC), and the majority of intra and extrahepatic cholangiocellular carcinomas. The rationale for exploring anti-CD73 treatments in patients with hepatobiliopancreatic cancers is supported by consistent CD73 expression. citing Sciarra A. et al. (2019)

2. CD73 and hepatic injuries

Additionally, ectonucleotidases can be expressed in the liver in a variety of cell types, such as endothelial cells and local immune cells. Variations in the organ homeostasis have a significant impact on the specific cellular localization and function, as was seen in the rat. In fibroblastic cells that lie beneath vascular endothelial cells and smooth muscle cells in healthy rat liver, CD73 expression partially combines with that of ENTPD1/CD39, and with that of ENTPDase8 in bile canaliculi. A fibroblast subpopulation in portal spaces that is close to ENTPDase2+ portal fibroblasts expresses CD73. Contrary to their normal, dormant states, these ectonucleotidases' expression and activity are significantly changed in fibrotic livers. [M. S. Longhi et al., 2019]

Ischemia/reperfusion injury (IRI) is brought on by the vascular damage that results from re-oxygenating tissues that have been deprived of oxygen. IRI is a condition that leads to platelet activation, organ rejection, and a buildup of inflammatory mediators, such as adenine nucleotides (see Table 3). Grafts are protected from ischemia damage by ENTPD1 expression in donor livers and high concentration adenosine therapy [Longhi M.S. et al, 2019].
Purinergic activation of immune cells and vascular endothelium has been connected to the inflammatory liver injury brought on by acetaminophen (APAP) toxicity [Longhi M.S. et al., 2019]. Patients with advanced liver failure still have only one therapy option: liver transplantation. Given the limited number of supporting options, it is imperative to prevent early graft malfunction, which is mostly caused by IRI. As a result of substantial hepatic infarction, CD73 defective mice are unable to be preconditioned and remain very vulnerable to the consequences of protracted ischemia. In 2014, [Dwyer K.M. et al.]

An intrinsic reaction to hepatic ischemia and reperfusion is inflammation, and T lymphocytes quickly build up within the liver parenchyma. A2AR activation on circulating cells was found to give protection with less liver damage, decreased neutrophil infiltration, and production of proinflammatory cytokine transcripts through a series of investigations employing chimeric mice. Lappas et al. further developed these data and showed that the NKT cell fraction of CD4+ T cells mostly mediates early liver damage. NKT cell activation, on the other hand, gives protection before the ischemic insult. When NKT cells were stimulated 1 hour before ischemia, Cao et al. observed a decrease in neutrophil infiltration and damage that was IL-13 and A2AR dependent [Dwyer K.M. et al, 2014].

8. Functions in the Cardiovascular system:

1. ROLE of ecto-5'-nucleotidase (CD73) IN CARDIOLOGY

CD73 activity and adenosine metabolism have both been shown to occur during short ischemia-induced cardiac preconditioning. Ecto-50-nucleotidase (CD73), which is activated during ischemia and hypoxia, is believed to be principally in charge of adenosine synthesis under those conditions. Myocytes may be the source of the rise in CD73 found in myocardial exposed to IP, according to some data. CD73's relative relevance in cardiac tissue has recently been shown [Colgan SP, 2006].

1. CD73 FUNCTIONS IN THE HEART

The following scientific evidence are established:

- CD73 expression and distribution in the heart
- CD73 protects in MI Injury
- CD73 protects in experimental heart failure

Olsson in 2004 gave a quick overview of some of the studies that helped to establish CD73 as a crucial component of the cardiovascular system. In their review, Burnstock and Pelleg emphasized further pertinent studies on purinergic signaling in the heart. Here, we concentrate on the most recent research establishing CD73's protective roles in myocardial infarction (MI) and heart failure as well as its cell type-specific functions in the cardiovascular system [Minor M. et al, 2019].

CD73 expression was found on smooth muscle cells, endothelial cells, and local lymphocytes in the cardiovascular system. However, there is a disparity in the expression of CD73 on cardiomyocytes, smooth muscle cells, and endothelial cells in the normal mouse heart according to certain published studies. While one study claimed that these cell types didn't express CD73 in the normal mouse heart, other animal studies and data from the Human Protein Atlas indicate that this protein is moderately expressed in these cell types under baseline circumstances [Minor M. et al., 2019]. This mismatch may be caused by the disruption of tissue architecture during digestion and processing for cell sorting, which increased mechanical signaling and caused nonimmune cells in the heart tissue to downregulate their surface CD73 expression. Kindlin-2, a mechanosensitive cytoskeletal protein that modulates integrin signaling in endothelial cells and cardiomyocytes and controls CD73 trafficking to the membrane, may have a role in CD73 downregulation during tissue digestion. Therefore, it is important to carefully analyze the experimental conditions that were employed to separate the cells when evaluating the expression and function of CD73 on cells isolated from the heart and other solid tissues [Minor M. et al, 2019].

Ischemia-reperfusion (I/R) injury during acute MI leads to myocardial injury. Following MI, T cell-expressed CD73, which reduces inflammation by producing adenosine, is essential for tissue healing and recovery. Particularly, following myocardial infarction, circulating T lymphocytes enter the damaged heart and increase the expression of hydrolyzing enzymes that act on ATP, cAMP, and NAD, leading to the synthesis of adenosine via CD73. Releasing of inflammatory mediators is decreased by activation of A2AR and A2BR, which signal via Gs proteins. Th1 and Th17-dominant T cells from Nt5e mice produce more of the proinflammatory cytokines IFN- and IL-17 than other T cell types. In keeping with this, in post-MI swine heart, monocytes cocultured with mesenchymal stem cells upregulate CD73 expression in vitro and in vivo, which supports an anti-inflammatory state and implicates CD73 in the healing actions of mesenchymal stem cells [Minor M. et al, 2019].

Importantly, after cardiac arrest (CA), which causes global I/R injury, human patients still exhibit the anti-inflammatory effects of CD73 that have been observed in animal models of myocardial injury. Improved survival after CA was linked to higher levels of CD73+ lymphocytes, possibly because to their anti-inflammatory properties. In particular, myeloid cells' in vitro production of proinflammatory stimuli (TNF- and ROS) was inhibited by CD73+ lymphocytes derived from CA patients. In 2019, Minor M. et al.

It's interesting to note that after MI, an elevation of CD73 that is functionally meaningful occurs on epicardium-derived cells (EPDCs), which encourages the production of pro-inflammatory cytokines and the profibrogenic matrix protein Tenascin-C. Following ischemic heart injury, EPDCs, which are ordinarily dormant in the adult heart, become active and give rise to numerous cell types. Unlike T cell responses, proinflammatory cytokines (IL-6, IL-11, and VEGF) are released by EPDCs in response to enhanced CD73-generated adenosine synthesis and A2BR activation. These investigations show that different cell types coordinate CD73 activities before, during, and after MI [Minor M. et al., 2019]. Global CD73 and CD4-CD73 animals can be made to experience myocardial ischemia, which causes tissue damage, T-cell purinergic signaling, cytokines, and cardiac dysfunction [Schrader J. et al, 2017]. Critical transporters and enzymes (connexin, pannexin, equilibrative nucleoside, CD73, ecto-nucleotide pyrophosphatase/phosphodiesterases, CD38) for the accelerated release and hydrolysis of ATP, cAMP, AMP, and NAD to adenosine can be significantly upregulated at the gene and protein levels by T cells infiltrating the injured heart [Schrader

In a time-dependent way, CD73 expression is increased in a variety of T cell types (cytotoxic, helper, and regulatory) in a mouse model of heart failure brought on by transverse aortic constriction (TAC). Since mice

lacking Nt5e globally, or only on T cells, show greater fibrosis and severe deficits in heart function after TAC, this overexpression appears to have a protective role. This is followed by an increase in the release of proinflammatory cytokines (IL-3, IL-6, and IL-13) from CD73-deficient T cells. As a result, CD73 on T cells controls excessive inflammation during TAC-induced heart damage, which is similar to the findings in the MI model [Minor M. et al, 2019]. T lymphocytes are necessary for effective myocardial infarction repair. However, it is unknown how they work to be useful. The ectonucleotidase CD73 converts the proinflammatory danger signal ATP, which is produced by injured cells, into the anti-inflammatory mediator adenosine. T lymphocytes produce CD73-derived adenosine to aid in cardiac remodeling during ischemia/reperfusion [Schrader J. et al, 2017].

Studies revealed that T cells and myeloid cells in the heart both have transcription of the A2b receptor (A2bR) increased after myocardial infarction. As a result, signaling by the A2aR and A2bR may influence how the myocardium reacts following a myocardial infarction. When CD73 was absent, T cells secreted proinflammatory and profibrotic cytokines (interleukin-2, interferon, and interleukin-17) more quickly than normal. A2aR activation reduced the ability of peripheral lymph node-derived T cells to produce cytokines (CGS-21680). BAY 60-6583, an A2bR agonist, had off-target effects. A particular A2bR antagonist blocked each of the effects of the adenosine receptor agonist NECA, which suppressed interferon- and promoted interleukin-6 synthesis [Schrader J. et al, 2017].

In response to the ischemia-induced death of cardiomyocytes, the injured heart is cleared of dead cells and matrix debris, and the damaged tissue is replaced by a stable scar. Damage-associated molecular patterns are released by injured cardiomyocytes, which activate innate immunity and cause the production of proinflammatory cytokines and chemokines, which further draw leukocytes. Monocytes and lymphocytes invade the heart later after neutrophils, who do so first. Innate immune system cells play a major role in the wound healing process required for the development of highly vascularized granulation tissue. Recent data, however, indicates that CD4+ T cells may also have an impact on the healing and scarring process. As evidenced by liver reperfusion injury, CD4+ invariant natural killer T cells may also contribute to inflammation following reperfusion [Schrader J. et al, 2017].

T cells play a significant role in cardiac remodeling, as studies have amply shown; nevertheless, the mechanisms behind T cells' ability to produce cardiac protection are still poorly understood [Schrader J. et al., 2017].

Global CD73 deficiency has been linked to a substantial impairment in heart function during ischemia/reperfusion (I/R), as well as a protracted inflammatory response and accelerated fibrosis. The present study used freshly created CD4-CD73 mice to investigate the function of CD73 on T cells on the cardiac healing process and ventricular remodeling because CD73 is mostly expressed on granulocytes and T cells after MI [Schrader J. et al, 2017].

T cells that invade a damaged heart go through a lot of purinergic metabolic reprogramming. In the third stage, they create adenosine via CD73, which by A2aR and A2bR suppresses in a feedback loop way the synthesis of significant proinflammatory (IFN-) and profibrotic (IL-17) cytokines. They upregulate their enzymatic machinery for the rapid hydrolysis of ATP, cAMP, and NAD. An effective illustration of the significance of adenosine in adaptive immunity for preventing negative ventricular remodeling after MI is provided by the fact that resolution of inflammation after MI significantly requires changes in extracellular purine metabolism on IL-17-secreting T cells. [J. S. Krader et al., 2017]

According to the current study, CD73 mice had altered T-cell polarization due to greater levels of Th1 T cells (which generate IFN- and IL-2) and Th17 cells, which are the main source of IL-17. Increased Th1 and Th17 T cell counts are recognized to contribute to tissue deterioration and to be important autoimmune mediators.

Particularly, IL-17 triggers a positive feedback loop that maintains the proinflammatory environment and the primary immune response, leading to excessive tissue damage [Schrader J. et al., 2017].

We have previously stated that cardiac APCs and cardiac fibroblasts lack CD73, are only capable of degrading nucleotides to the level of AMP, and need T cells and granulocytes that express CD73 in order to degrade nucleotides further to adenosine. granulocytes that produce adenosine and positions CD73 on T cells in the center of the metabolic chain. As a result, in addition to hydrolyzing AMP that is delivered to T cells via diffusion from nearby APCs and fibroblasts, T cells also destroy the ATP and NAD produced following activation. According to this theory, T cells take part in AMP's final transformation into adenosine. In addition to the A2aR's well-known anti-inflammatory action, which has been shown in numerous cellular systems. As a result, crucial processes that trigger tissue repair and the resolution of inflammation seem to be under the control of adenosine [Schrader J. et al., 2017]. The metabolic pathways that alter when a T-cell is activated are closely related to how well they perform. Adenosine synthesis by granulocytes, the only other immune cell subpopulation expressing CD73 in the wounded heart, thus appears to be less significant because global CD73 and CD4-CD73 animals demonstrated an equivalent phenotype after MI [Schrader J. et al, 2017].

The genetic mechanism of the link between arterial calcifications and higher cardiovascular risk is uncertain [St. Hilaire C. et al., 2011]. In three families with symptomatic artery calcifications, we conducted clinical, radiological, and genetic analyses. There were transduction rescue tests, single-nucleotide-polymorphism analyses, targeted gene sequencing, quantitative polymerase-chain-reaction assays, Western blotting, enzyme measurements, and in vitro calcification assays carried out [St. Hilaire C. et al, 2011]. Vascular calcification, which can develop in a vessel's intima or media, is linked to an increased risk of cardiovascular events. This was once thought to be a passive reaction to degenerative processes, but accumulating evidence points to a process that mimics active bone remodeling as the cause. Extracellular calcification is increasingly believed to result from a default metabolic pathway and has to be prevented by continuously stimulating inhibitory mechanisms [St. Hilaire C. et al, 2011].

On the surface of numerous cell types, CD73 takes part in the extracellular pathway that transforms ATP into adenosine. Enpp1 deficiency directly causes lower pyrophosphate levels in patients with generalized arterial calcification of infancy, resulting in early-onset vascular calcification, myocardial infarction, and frequently infant mortality. Although pyrophosphate levels may not be directly affected by CD73 deficiency, the extracellular adenosine levels that are lowered as a result appear to increase TNAP activity; adenosine supplementation restored the increase in TNAP activity in CD73-deficient cells. The specific distribution of adenosine receptors in these organs may explain the lower-extremity arteries' preferential participation. In 2011, St. Hilaire C. et al.

Consideration of therapeutic approaches is made possible by understanding the fundamental flaw in our patients. Patients with CD73 deficiency may benefit from the use of bisphosphonates, which are pyrophosphate analogues and powerful tissue calcification inhibitors. These drugs have been used successfully to treat ENPP1 deficiency. Since dipyridamole inhibits cellular absorption of adenosine (and subsequent breakdown by adenosine deaminase) both in vitro and in vivo, it may be able to give adenosine rescue in patients with aneurysmal vascular remodeling. 25 Adenosine-receptor agonists and direct inhibitors of TNAP, such as lansoprazole, are further treatment options. 26,27 Cultured cells that exhibit both TNAP and calcification characteristics that are eliminated by transduction with a CD73-encoded lentiviral vector can be used to test the potential effectiveness of such therapies. If a calcification phenotype can be identified, CD73-deficient mice can also be used to study the role of adenosine in controlling vascular calcification, affecting bone mineralization, and modulating ectopic calcium deposition. NT5E mutations in members of three families who had symptomatic calcifications of the joints and arteries. This gene produces the nucleotidase CD73, which turns AMP into adenosine. Our findings therefore suggest that this metabolic route inhibits the calcification of ectopic tissue. In 2011, St. Hilaire C. et al.

High expression of a differentiation cluster Cardioprotective benefits are provided by CD73 [St. Hilaire C. et al, 2011]. According to Kim S-H. et al. (2018), open heart surgery (OHS) with aortic cross clamping causes ischemia-reperfusion damage (IRI) and a systemic inflammatory response, leading to serious postoperative consequences. Previous research demonstrated that CD73 protects organs from IRI. Kim et al. demonstrated that CD73 protects renal IRI, and Bonner et al. demonstrated that myocardial protection against cardiac IRI is provided by CD73 up-regulation (see Table 3). Additionally, by preventing the production of adenosine after IRI, blocking CD73 may cause organ damage. Aortic cross clamping is required for several hours during OHS [Kim S-H. et al., 2018].

Propofol and sevoflurane showed comparable effects on myocardial ischemia and surgical complications, according to Lurati Buse et al. Additionally, a recent extensive meta-analysis demonstrated no distinction between propofol and volatile anesthetics in terms of survival. The recent consideration of increased CD73 expression in animal models as a potential treatment strategy to prevent inflammatory reactions against IRI Therefore, reducing IRI-related inflammation and surgical consequences may be achieved by modulating CD73 expression by modifying propofol dosage. Examining CD73's effects in relation to various anesthetics may be difficult in the clinical context because prior investigations of its anti-immunosuppressive effects used animal models [Kim S-H. et al, 2018].

Triggering of CD38 can boost lymphocyte CD73 expression. In this instance, more CD73 is transferred from an intracellular pool to the cell surface. Given that IFN- took a considerable amount of time to up-regulate CD73 in vitro, it is conceivable that this is also true of CD73's up-regulation at inflammatory sites in vivo. It may also suggest that the body's innate defense mechanisms for reducing and limiting inflammation include up-regulation of CD73 and enhanced adenosine synthesis. This is consistent with research showing that adenosine protects cells against ischemia-induced cell death in the heart and central nervous system. Ecto-5′-nucleotidase activity rises after hypoxia as a result of preconditioning. Large levels of adenosine are released as a result, increasing the cells' resistance to infarction, as in cardiac hypoxia. [Joe Niemelä et al., 2004]

2. Cardiac Transplantation

Endometrial regenerative cells (ERCs), a recently discovered cell type, have been shown to promote immunological tolerance in cardiac allograft transplantation. The expression of Ecto-5′-nucleotidase (CD73) on ERCs is essential for the preservation of cardiac allografts. Studies revealed that CD73 expression was essential for the reduction of graft disease caused by ERC. The percentage drop of tolerogenic dendritic cells, macrophage type 2 (M2), and regulatory T cells was correlated with the inhibition of CD73 expression on ERCs (T-regs). When compared to the group of ERCs that had not been primed with anti-CD73 mAb, CD73-expressing ERCs considerably boosted the amount of the anti-inflammatory cytokine IL-10 while significantly reducing the level of pro-inflammatory cytokines including IFN and TNF. Furthermore, ERCs that expressed CD73 demonstrated tissue protective function by controlling the expression of the adenosine receptor, which was connected to the infiltration of CD4+ and CD8+ cells in the allografts. Additionally, a considerable increase in A2B receptors in the cardiac allograft was linked to a CD73-mediated extension of cardiac allograft survival that was caused by ERC. In 2020, [Wang H. et al.]

Organ transplantation is currently the last option for many terminal conditions that are life-threatening. Nearly 35000 patients in the US received donated organs just in 2018. Rejection of the allograft remains the main barrier to a successful transplant, nonetheless. Although the development and use of immunosuppressive medications has substantially increased the length of time that grafts can survive, it is important to be aware of their drawbacks, which include chronic graft malfunction, infection, and cancer. Finding a better, safer, and more efficient immunosuppressive medication is urgently needed in these circumstances. In 2020, [Wang H. et al.]

Multipotent stem cells, such as mesenchymal stem cells (MSCs), can differentiate into a variety of cell types, including adipocytes, osteoblasts, and chondrocytes. Data indicated that MSCs were helpful in treating a number of ailments, including autoimmune disorders, tissue damage, graft rejection, and others. MSCs have been shown to have a role in the field of transplantation by inducing immunological tolerance in animal models and showing promise for therapeutic use. However, the challenges for using MSCs in clinical settings in large numbers continue to include the invasive harvesting process, constrained capacity for proliferation, and decreased availability. In 2020, [Wang H. et al.]

A prospective immunoregulatory protein known as ecto-5'-nucleotidase (CD73) can be expressed by a variety of cells, including B cells, T cells, neutrophils, natural killer cells, monocytes, and macrophages. Additionally, CD73 is essential for purinergic signaling, the enzyme that limits the production of extracellular adenosine (ADO). In addition, the ADO can bind to the A1 receptor, A2A receptor, A2B receptor, and A3 receptor. As a result, CD73 not only exhibits biological capabilities on its own, but also produces effects when combined with ADO receptors. For instance, CD73 loss in either donors or recipients reduced graft survival in a heterotopic cardiac allotransplantation paradigm. In the meantime, it has been demonstrated that the CD73-ADO pathway, by binding to the A2A and A2B receptors, prevents excessive immune responses and reduces immune-mediated tissue damage in the context of anti-tumor therapy [Wang H. et al., 2020].

On the surface of ERCs, the multifunctional CD73 is highly expressed. Resta et al. have also shown that the murine protein is strikingly comparable to the human form and that murine CD73 is 94% identical to human CD73 at the amino acid level. We hypothesize that CD73 expression is essential for ERCs-mediated cardiac allograft protection given its immunoregulatory characteristics and CD73 expression [Wang H. et al., 2020].

The best treatment option for some terminal conditions today is organ transplantation. Despite the fantastic future of organ transplantation, there are still two significant obstacles to be overcome: the lack of donor organs and immunological rejection. On the one hand, efforts should be undertaken to expand the pool of organ donors available while addressing the two challenging issues. On the other hand, more study is required to determine how to prevent immunological rejection and increase the duration the graft survives in order to lower the demand for organs. Our earlier research and the current study also demonstrate that ERCs can increase cardiac transplant survival. Even long-term survival is possible when rapamycin and ERC-based therapy are combined. As a result, it suggests that ERCs have the potential to act as immunosuppressive agents [Wang H. et al, 2020].

An increasing amount of research has shown that CD73, a major purinergic signaling enzyme that converts AMP to ADO, plays a role in a number of physiological and pathological processes, including the mediation of ERC-induced cardiac allograft protection. In addition to being expressed on ERCs, CD73 is generally present in many different tissues and cells, such as the tissues of the heart and immune cells. More critically, there are three effective ways to block CD73's ability to operate. In this heart transplant model, the CD73 inhibiting ERCs also shown a significant reduction in immunoregulation and graft protection [Wang H. et al, 2020].

It is well established that A2AR activation prevents CD4+ cell infiltration in the reperfused heart, which has many positive effects in the treatment of coronary occlusion. The protection of cardiac allografts may be influenced by the high expression of A2B receptors. In 2020, [Wang H. et al.] The growing body of evidence demonstrated the therapeutic effects of human-derived stem cells in several xenogeneic models due to the homology between human CD73 and mouse CD73 [Wang H. et al, 2020].

Three factors contributed significantly to the protection of the cardiac allograft:

1) In the first place, CD73 expression affected the immune system and caused it to change in a way that protected the graft.

2) In addition, ERCs that express CD73 have higher levels of anti-inflammatory cytokines and lower levels of pro-inflammatory cytokines.

In addition to performing tissue protection, CD73 expression on ERCs also controlled the tissue expression of ADO receptors. In order to achieve long-term allograft acceptance, increasing CD73 expression may help ERCs perform their immunoregulation role [Wang H. et al., 2020].

Coronary Allograft Vasculopathy (CAV), a quickly progressing form of atherosclerosis that causes decreased blood flow and ischemia, is a common cause of mortality in patients who survive more than a year after receiving a heart transplant. It is a symptom of chronic cardiac allograft rejection. Although many immune-mediated and metabolic risk factors have been linked to the pathogenesis of CAV, there is currently no viable cure for the disease or the unfavorable effects it is associated with. The primary therapy approach for CAV focuses on mitigating and treating the risk factors that are known to cause or hasten the disease, such as recurrent bouts of acute allograft rejection and extended cold ischemia time linked to severe IRI (see Table 3). In 2014, [Dwyer K.M. et al.]

Table 3: Expression of the ectonucleotidases and adenosine receptors on the organ parenchyma during ischemia–reperfusion injury.

Organ parenchyma	Ectonucleotidase and adenosine receptors critical in IRI
Heart	CD39, CD73, $A_{2B}R$
Trachea (lung)	$A_{2A}R$
Liver	CD39, CD73
Kidney	CD39, CD73, $A_{2B}R$

In order to mediate cardioprotection against hypoxic damage, the A2BR is crucial. Early after engraftment, 4 hours after heart transplantation, it has been demonstrated that the A2BR rises and changes in CD73 mRNA expression coincide. In a mouse model of cardiac transplantation, CD73 deficiency in either the donor or the recipient decreased graft survival, accelerated the development of CAV, and was linked to decreased A2BR expression. Under situations of myocardial hypoxia, the expression of CD73 and A2BR is coordinated and reliant [Dwyer K.M. et al, 2014]. When the donor heart is transported to the recipient center, it must undergo a lengthy period of cold preservation while being kept on ice. An adenosine bolus administered prior to cardioplegia and storage in a rat heterotopic heart transplant model lowered myocardial damage and accelerated reanimation after reperfusion. Adenosine treatment resulted in less inflammation and fewer infiltrating cells in the grafts [Dwyer K.M. et al, 2014].

In a model of myocardial ischemia and reperfusion, Bonner et al. recently showed that CD73 on circulating immune cells was essential for heart repair. After 3 days of ischemia and reperfusion, granulocytes and T lymphocytes, which strongly express CD73, had penetrated the myocardium. Limiting infarct size, inflammation, and fibrosis development required the production of adenosine. While infarct size and cardiac ejection fraction were comparable the day after ischemia, CD73 defective mice's cardiac function continued to deteriorate while WT mice's cardiac function showed some signs of recovery. A prolonged leukocytic cardiac infiltration with a Th1 and M1 phenotype and increased production of TNF, IL-1, IL-6, and IL-17 was linked to a lack of CD73. Through A2BR signaling exclusively on polymorphonuclear cells, Koeppen et al. showed that CD73-generated adenosine reduced inflammation and fibrosis while retaining heart function [Dwyer K.M. et al., 2014].

9. Functions in the Nervous system:

1. Central nervous system (CNS)

The CNS functions of locomotion and behavior, memory and plasticity, sleep regulation, thermoregulation, host-pathogen interactions during brain infection, inflammation, and nociception have all been linked to CD73 in several studies. We have highlighted many research [Minor M. et al., 2019] that describe both novel and well-established processes of CD73 in the brain and spinal cord.

According to assessments, the following tasks are fundamental:

- ☐ CNS CD73 Expression and Distribution
- ☐ CD73 Regulates Motion
- ☐ Thalamic CD73 Blocks Plasticity of the Auditory Cortex
- ☐ CD73 Mediates Inflammation of the CNS
- ☐ CD73 Regulates Nociception in Neurons

Astrocytes also exhibit high levels of CD73 expression, which may regulate astrocyte behaviors like migration and damage responses. It is closely related to the A2AR in the postsynaptic compartment of the striatum. This intimate contact seems crucial for regulating movement. With other ectonucleotidases, particular adenosine receptor subtypes, and CD73, the effect of CD73 on locomotion appears to be context-specific and presumably subject to the spatiotemporal dynamics of the signaling pathways under its control. M. Minor et al., 2019.

Numerous studies have focused on the role of CD73 in brain inflammation because of the important part that adenosine plays as an immunomodulator. In particular, Nt5e mice had a higher risk of suffering an ischemic stroke injury, and ischemic tissue had higher levels of macrophage influx and activation as well as proinflammatory markers as IL-1, IL-6, and TNF-. Administration of soluble CD73 abolished this effect, indicating that it was adenosine-mediated. M. Minor et al., 2019.

Additionally, bone marrow transplantation studies showed that CD73 had a protective impact on tissue-resident cells rather than circulating immune cells that entered the area following the damage. Although the precise function of CD73 on astrocytes has not been studied, it is probable that astrocytes are crucial to this model since they produce adenosine in response to inflammation and regulate neuronal damage after ischemic stroke. Similar to the brain, the heart, liver, and kidneys have also been shown to be protected by CD73 following ischemic tissue injury. M. Minor et al., 2019.

According to Minor M. et al. (2019), CD73 encourages macrophages and microglia to adopt the M2 anti-inflammatory phenotype. This suggests that immune cells may be partially responsible for CD73's protective function in pain models.

1. *CD73 and adenosine in the nervous system*

Scientific proof has revealed the following:

- ☐ CD73 Is a Major 5'-Nucleotidase Generating Adenosine in the Mouse Brain • CD73 Is a Major Regulator of Adenosinergic Signaling in the Mouse Brain
- ☐ CD73 controls exploratory locomotion
- ☐ Social behavior modification in CD73-deficient mice
- ☐ In CD73-deficient mice, sensory and memory capabilities were preserved.

The cell surface enzyme CD73 (ecto-5'-nucleotidase) is known to control purinergic signaling by desphosphorylating extracellular AMP to adenosine. Although it is known that 5'-nucleotidases are expressed in the brain, it is still unknown whether CD73 is expressed there or if it has any potential physiological use. We discovered that CD73 is strongly expressed in the basal ganglia core, which is made up of the striatum (caudate nucleus and putamen) and globus pallidus, using immunohistochemistry on wild-type and CD73 deficient mice [Kulesskaya N. et al., 2013]. Furthermore, it was shown that CD73 is selectively expressed in the olfactory tubercle and meninges. Analysis of mice with CD73 deficiency and wild-type (wt) mice showed that CD73 confers the majority of 5'-nucleotidase activity in a number of brain regions. The CD73 defective mice showed dramatically increased exploratory locomotor activity in a variety of behavioral tests and IntelliCage experiments, which is likely due to the strong expression of CD73 in the striatum and globus pallidus, which are known to regulate locomotion. The mice lacking CD73 also showed impaired social behavior. Adenosinergic signaling in the brain is important in the control of normal and abnormal behavior, and our studies offer a fresh mechanistic insight into this process [Kulesskaya N. et al., 2013].

The extracellular nucleotide breakdown is significantly regulated by this ecto-5'-nucleotidase. Adenylate kinase and CD73 are two enzymes that can hydrolyze extracellular AMP to produce adenosine or convert it to ADP. Adenosine can bind to four distinct receptors: A1, A2A, A2B, and A3; these receptors allow it to have numerous signaling effects in a variety of tissues. In 2013, Kulesskaya N. et al.

Adenosine is an essential component of the central nervous system (CNS), which regulates a wide range of brain activities. Adenosine is engaged in a variety of physiological and pathological processes through the activation of its G-protein coupled receptors, including the control of sleep, general arousal state and activity, local neuronal excitability, and coupling of cerebral blood flow to the energy need. Additionally, adenosine signaling modification may have therapeutic potential in the treatment of psychiatric disorders like schizophrenia and autism as well as neurodegenerative diseases like Alzheimer's, Parkinson's, and Huntington's diseases. As a result, changes in adenosine concentrations may have a significant impact on how the entire organism behaves and performs [Kulesskaya N. et al, 2013].

The actions of CD73 at that region are nearly completely unknown, in contrast to the abundance of knowledge on the function of adenosine in the central nervous system. Since there are seven different 5'-nucleotidases in the human body, it is significant to note that utilizing gene-deficient mice is the only way to essentially unravel how CD73 contributes to the generation of adenosine. In numerous organs, including the diseased brain, CD73 has been demonstrated to control the balance of vascular permeability and inflammation. In 2013, Kulesskaya N. et al.

Unknown is CD73's function in regulating behavioral characteristics. There is a contribution of CD73 to the purinergic signaling cascades' enzymatic activity in the brain, and CD73 now has novel roles in regulating social relationships and exploratory behavior. In 2013, Kulesskaya N. et al.

According to enzyme cytochemistry, 5'-nucleotidases are mostly linked with glial cells in the adult nervous system, although they are active in the synaptic cleft during development and regeneration. The subcortical regions of the brain parenchyma, particularly the striatum and globus pallidus, contain the CD73 protein. According to Kulesskaya N. et al. (2013), CD73 confers the majority of the 5'-nucleotidase activity in the brain in CD73-deficient animals. Pharmacological or genetic methods have indicated that adenosine is implicated in the control of wakefulness, sleep, learning and memory, fear, anxiety, and motor activities. In 2013, Kulesskaya N. et al.

Since adenosine is a primary 5'-nucleotidase that generates adenosine in the brain, the observation of hyperactivity in CD73 defective mice fits well with the discovery that adenosine is a worldwide regulator of neuronal activity in the brain, causing a basal inhibitory tone of behavioral activity. Thus, the absence of CD73 results in a deficit in adenosine-dependent signaling that cannot be made up for by other brain-based 5'-nucleotidases. High levels of CD73 expression in the striatum point to A2A receptor involvement in mediating the observed locomotor phenotype. Animal models have shown that adenosine A2A receptor antagonists have motor effects, and the striatum exhibits significant interactions between adenosine and dopamine receptors that affect spontaneous locomotor activity [Kulesskaya N. et al., 2013].

Adenosine is a neuromodulator that acts through facilitatory A2ARs and inhibitory A1Rs, which have affinities for adenosine that are comparable. It has been demonstrated that intracellular adenosine kinase activity primarily regulates the activation of A1Rs, but it is uncertain where the adenosine that activates A2ARs comes from. The primary enzyme that transforms extracellular AMP into adenosine, cto-5'-nucleotidase (CD73), colocalizes with A2ARs in the basal ganglia. In addition to astrocytes, postsynaptic locations are a key location for striatal CD73. Notably, proximity ligation experiments demonstrated the close vicinity of CD73 and A2ARs in the striatum and CD73 coimmunoprecipitated with A2ARs. As a result, cAMP production in synaptosomes and hypolocomotion brought on by a novel A2AR prodrug, which activates A2ARs by metabolizing CD73, were only seen in wild-type mice and not in CD73 knockout (KO) or A2AR KO animals. Additionally, CD73 KO mice showed improved working memory abilities and a reduced sensitivity to amphetamine-induced sensitization, reflecting the phenotype of global or forebrain-A2AR KO mice as well as that observed upon pharmacological A2AR blockage. These findings demonstrate that striatal A2AR function is activated by CD73-mediated extracellular adenosine production. According to this study, CD73 is a unique therapeutic target that can be used to alter A2AR-mediated modulation of striatal function and neurodegeneration [Augusto E. et al., 2013]

Adenosine primarily modulates brain neurotransmission through inhibitory A1 receptors (A1Rs) and facilitatory A2 receptors. In the brain, A1Rs are widely expressed and regulate synaptic transmission. Enhancing A1R activation through regulation of adenosine kinase provides neuroprotection against brain damage caused by glutamate excitotoxicity, namely during epilepsy and brain ischemia, in agreement with their inhibitory role in reducing excitatory transmission (Fredholm et al., 2005). Importantly, controlling neurodegeneration through the manipulation of metabolic pathways linked to A1R activation is more promising than doing so directly because the former locally increases adenosine where activity is disrupted while the latter also activates peripheral A1R, which has noticeable cardiovascular effects. citing Augusto E. et al. (2019)

The sole enzyme in the brain capable of dephosphorylating extracellular AMP into adenosine is ecto-5'-nucleotidase (CD73), which limits and regulates the ATP-derived synthesis of adenosine by ecto-nucleotidases (Cunha, 2001b) (Lovatt et al., 2012). According to this postulated functional relationship between CD73 and A2ARs, CD73 activity exhibits a brain distribution that is comparable to that of A2ARs, both of which are more

prevalent in the basal ganglia. According to Augusto E. et al. (2013), CD73 provides the specific pool of extracellular adenosine that is specifically responsible for activating striatal A2ARs.

The last enzymatic step in the production of extracellular ATP-derived adenosine is carried out by CD73, which plays a critical role in the activation of striatal A2ARs. CD73 appears to be the primary enzyme dephosphorylating AMP to adenosine in the CNS. Their anatomical location and close proximity in the striatum suggest the inherent relationship between CD73 and A2ARs. Similar distribution patterns of CD73 and A2ARs in the basal ganglia, as well as the enrichment of these two molecules at postsynaptic sites and their close proximity, all point to their colocalization in the striatum. In 2013, Augusto E. et al.

In vivo, three significant behavioral responses that have previously been shown to involve A2AR activation—hypolocomotion, decreased working memory, and behavioral sensitization to psychoactive drugs—further supported the functional relationship between CD73 activity and the activation of striatal A2ARs (Yu et al., 2008). Since CD73 KO mice exhibit a normal A1R-mediated regulation of synaptic transmission, they have lower levels of adenosine in general (Zhang et al., 2012). Without affecting the basic form of locomotion, inactivating A2ARs eliminates the psychomotor sensitization to amphetamine [Augusto E. et al, 2013].

It was also demonstrated that inactivating A2ARs improves working memory function, a similar phenotype to that seen currently following the administration of an A2AR antagonist, as well as in CD73 KO mice, but not after the administration of an A1R antagonist. The ability of A2ARs to regulate synaptic plasticity in hippocampal synapses or synaptic adaptation at the neuromuscular junction is specifically muted by the suppression of CD73 (Rebola et al., 2008). Furthermore, it has been demonstrated that the activity of CD73 is definitely necessary for the control of vascular tone and the immune-inflammatory system by A2ARs. The finding that a number of circumstances cause a coordinated induction or suppression of CD73 and A2AR expression is further evidence of the close relationship between CD73 and A2ARs and strongly supports the idea that these two molecules are interrelated [Augusto E. et al., 2013].

Notably, rather than the more prevalent inhibitory A1Rs in the nervous system, CD73-mediated synthesis of adenosine appears to selectively associate with the activation of facilitatory A2ARs. The distinct location of CD73 and A1Rs across the brain further supports the separation between CD73 activity and A1R activation. However, it cannot be completely ruled out that ATP-derived adenosine may also activate A1Rs in specific systems, such as in the regulation of tubuloglomerular feedback (Thomson et al., 2000) or of nociception, which calls for the involvement of alkaline phosphatase, which we have now ruled out to be a factor in the extracellular catabolism of AMP in striatal synapses [Augusto E. et al, 2013].

To explain the differential activation of inhibitory A1Rs and facilitatory A2ARs in accordance with the functional requirements of neural circuits, this selective activation of A2ARs by CD73-mediated adenosine production lends direct support to the earlier hypothesis (Cunha, 2008). which is regulated by A2ARs in synapses (Duarte-Pinto et al., 2005) may also be involved in preventing CD73-generated adenosine from activating A2ARs, as was recently suggested [Augusto E. et al, 2013].

It is also unclear if the correlation between the activation of A2ARs under near-physiological conditions and the production of ATP-derived adenosine by CD73 can be generalized to diseased brain conditions. Indeed, A2AR blockage is known to provide strong neuroprotection in animal models of disorders of the brain, including epilepsy, ischemia, Alzheimer's, and Parkinson's diseases (Chen et al., 1999). It is enticing to think that manipulating CD73 would provide benefits comparable to those seen with A2AR blockage [Augusto E. et al, 2013]. When combined with the hypothesis that glial A2ARs play a role in neurodegeneration, this could potentially provide a functional explanation for the localization of CD73 in astrocytic membranes, which is now also verified to be present in gliosomes (Yu et al., 2008)

2. CD73 and T cells, T lymphocytes in the nervous system

A distinct subset of immunosuppressive T cells called regulatory T (T-reg) cells is crucial for maintaining immunological homeostasis. In numerous pathological conditions such as autoimmunity, injury, and nervous system degeneration, they preserve self-tolerance, prevent autoimmune, and serve as essential negative regulators of inflammation. According to growing studies, a variety of peripheral and central nervous system diseases may affect how T-reg cells behave. T-reg cells are known to have both positive and harmful effects in specific disease circumstances [Kulesskaya N. et al, 2013]. There is growing proof that T-reg cells play a dual role in the development of neurological disorders such multiple sclerosis, Guillain-Barré syndrome, neuropathic pain, stroke, and neurodegenerative illnesses like amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease. The significance of T-reg cells in these disorders is still being fully uncovered, and gaining a deeper understanding of how these cells function in the neurological system will help us create fresh therapeutic approaches [Duffy S.S. et al., 2017].

Studies have shown that regulatory T cells play either beneficial, harmful, or unclear functions in diseases of the nervous system, depending on the condition. While suppressing damaging immune responses and maintaining self-tolerance, regulatory T cells may also block effector T cell responses that work to stop ongoing neurodegeneration [Duffy S.S. et al., 2017]. Adenosine receptor-mediated signaling is impacted by the cell surface enzyme CD73, which regulates immunological and inflammatory responses. So it's crucial to look at how it expresses itself and where cells are located in the inflamed human peripheral nerve. There were no variations in CD4+ and CD8+ T cell counts between acute and chronic inflammatory polyneuropathies that stained positive for CD73. The peripheral nerve's endothelial cells did not express CD73. Our results provide credence to the idea that lymphocyte entrance into the inflamed nervous system is facilitated by CD73 [Kieseier B.C. et al, 2009].

3. CD73 and neuro-inflammation

The following scientific indications are demonstrated:

- ☐ CD73 expression and activity are significantly increased in white matter lesions
- ☐ CD73 deficiency exacerbated cerebral hypoperfusion-induced cognitive impairment
- ☐ CD73 deficiency induced more severe white matter rarefaction and glial activation
- ☐ CD73 deficiency significantly increased the levels of proinflammatory cytokines

Due to increased adenosine triphosphate (ATP) degradation during cellular stress or the release of extracellular ATP during cell death, which can be converted to adenosine by membrane-bound ecto-enzymes like CD39 and CD73, conditions of inflammatory tissue distress are linked to high extracellular levels of adenosine. In experimental arthritic models, the adenosine A2a receptor (A2aR) is known to mediate adenosine's anti-inflammatory actions. Here, we examined the functions of A2aR in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, utilizing pharmacological treatments and genetic inactivation (MS) 2016 [Ingwersen J. et al]

In the central nervous system (CNS), A2aR is upregulated in EAE; it is primarily seen on T cells and macrophages/microglia in the inflamed tissue. Application of the same agonist after the commencement of the disease increased non-remitting EAE progression and led to more severe tissue degradation. Preventive EAE treatment with an A2aR-specific agonist reduced myelin-specific T cell proliferation ex vivo and improved disease. Accordingly, faster and aggravated disease manifestation with higher frequencies of IFN-, IL-17, and GM-

CSF-producing CD4+ T helper cells and more inflammatory lesions in the early stage were observed in A2aR-deficient animals. In vitro, activation of A2aR reduced migration of CD4+ T cells, macrophages, and primary microglia as well as the phagocytosis of myelin by macrophages and primary microglia. However, EAE swiftly improved and myelin debris accumulation was decreased in A2aR mice. 2016 [Ingwersen J. et al]

The purine nucleoside adenosine is a modulatory messenger involved in numerous physiological and pathological immunological and central nervous system processes (CNS). High extracellular levels of adenosine are related to tissue damage conditions. The production of large amounts of extracellular ATP as a result of tissue damage and cell death, which can then be converted to adenosine by membrane-bound ecto-enzymes like CD73, is another possible source of adenosine. Intracellular adenosine triphosphate (ATP) degradation upon cellular stress, particularly under high energy demand, is also a source of adenosine. A damage-associated molecular pattern (DAMP) is influenced by increased extracellular concentrations of adenosine, which can be viewed as a general damage signal [Ingwersen J. et al, 2016].

According to Ingwersen J. et al. (2016), A2aR activation has a complex pattern in chronic autoimmune neurodegeneration. While it protects against inflammation by having anti-inflammatory effects on T cells early in the course of the disease, A2aR later in the course of the disease appears to be detrimental, which may lead to sustained tissue damage within the inflamed CNS. ATP catabolism mediated by ectonucleotidase offers a potent way to regulate extracellular adenosine levels. Although increased adenosine A2A receptor (A2AR) signaling in Parkinson's disease mice and patients has been well-documented, the cause of this elevated adenosine signaling is still unknown. Specifically, we demonstrate that upregulated CD73 and A2AR in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson's disease models cooperatively contribute to the elevated adenosine signaling. Ecto-5′-nucleotidase (CD73)-mediated adenosine formation provides an important input to activate A2AR. We also show that microglial immunoresponses and morphological dynamics are modulated by CD73-derived adenosine-A2AR signaling [Meng F. et al., 2019].

Although CD73 inactivation increased microglia process extension, mobility, and morphological transformation in the laser damage and acute MPTP-induced Parkinson's disease models, it greatly reduced lipopolysaccharide-induced pro-inflammatory responses in microglia. In Parkinson's disease mice, reducing CD73-derived adenosine significantly reduced microglia-mediated neuroinflammation and enhanced the survival of dopaminergic neurons and motor behaviors. Additionally, depletion of adenosine analogues reversed the effects of CD73 inactivation on A2AR induction and A2AR-mediated pro-inflammatory responses, suggesting that CD73 generates a self-regulating feed-forward adenosine formation to activate A2AR and promote neuroinflammation [Meng F. et al., 2019]. The findings suggest that the homeostatic balance between adenosine and dopamine signaling is crucial for microglia immune responses because A2A is known to exacerbate inflammation by inhibiting dopamine-mediated anti-inflammation. Thus, our research demonstrates a unique function for CD73-mediated nucleotide metabolism in controlling neuroinflammation and establishes the feasibility of targeting nucleotide metabolic pathways to reduce adenosine synthesis and neuroinflammation in Parkinson's disease. [Meng F. et al., 2019]

Numerous neurodegenerative disorders are accelerated or made worse by microglia-mediated neuroinflammation. Alzheimer's and Parkinson's illnesses, among others, have pathological characteristics of persistent microglia activation and significant neuroinflammation (Wang et al., 2015). The use of the neural-immune and neuroinflammation-related machinery to stop the progression of disease has shown promising results in the treatment of disease [Meng F. et al., 2019]. Extracellular adenosine is crucial for controlling the neuroinflammatory reactions caused by microglia (Hasko et al., 2005). In experimental autoimmune encephalomyelitis, A2AR inactivation has been demonstrated to intensify microglial inflammatory responses and aggravate disease development (Mills et al., 2012; Yao et al., 2012). In contrast, some studies have demonstrated that A2AR antagonism lowers the pro-inflammatory profile of microglia in brain injury models and inhibits lipopolysaccharide (LPS)-induced microglia activation (Rebola et al., 2011). It is unclear whether and how

adenosine synthesis is controlled to prevent A2A activation and microglia-mediated neuroinflammation in neurodegenerative illnesses [Meng F. et al., 2019].

A2AR on microglia may be activated by disease-sensitive CD73-produced adenosine to encourage neuroinflammation and the progression of Parkinson disease (Furman et al., 2017). A possible therapeutic approach for Parkinson's disease involves limiting adenosine synthesis and neuroinflammation by targeting nucleotide metabolic pathways. To further confirm the role of adenosine signaling in the disease, it would be beneficial to evaluate the long-term effects of the CD73-derived adenosine in chronic Parkinson's disease models [Meng F. et al, 2019]. Model showing how A2AR signaling and CD73-produced adenosine production contribute to neuroinflammation and neuronal degeneration mediated by microglia [Meng F. et al., 2019]

A fresh essential notion in the comprehension of the adaptive modifications of the adenosine modulation system in brain illnesses is implied by the current observation of a parallel elevation of CD73 and of A2AR in the Parkinson's disease model. In fact, the maladaptive neurochemical and behavioral changes that are typical of Parkinson's disease appear to be triggered by a concurrent increase in both A2AR and ATP-derived extracellular adenosine [Meng F. et al., 2019]. Our findings also suggest that blocking CD73-mediated extracellular adenosine formation prevented A2AR elevation in microglia in response to LPS-stimulation or MPTP toxicity in addition to inhibiting downstream A2AR signaling. This suggests an adenosine-dependent positive regulatory loop to upregulate A2AR and maximize the downstream pro-inflammatory effects. It is suggested that targeting CD73 to decrease adenosine availability offers a promising strategy to combat the A2AR-promoted neuroinflammation and to improve neuroprotection in the treatment of Parkinson's disease because this places CD73 at the center of coordination of the alterations of adenosine signalling in brain diseases [Meng F. et al, 2019].

Recent research has shown that immunological and inflammatory processes are crucial to the development of white matter lesions brought on by chronic cerebral hypoperfusion (CCH) (WMLs). The extracellular levels of adenosine, an endogenous neuromodulator in the brain, represent a crucial endogenous mechanism for the control of immunological and inflammatory responses. The rate-limiting step in the production of extracellular adenosine is thought to be catalyzed by the enzyme ecto-5’-nucleotidase (CD73), which dephosphorylates AMP to adenosine [Zheng J. et al, 2015].

An important factor in the development of spinal cord damage is immune activation, specifically stimulation of local microglia and macrophages that results in inflammation (SCI). Both the classically activated M1 phenotype, which has pro-inflammatory effects, and the alternatively activated M2 phenotype, which has anti-inflammatory effects, are forms of macrophages/microglia. Ecto-5’-nucleotidase (CD73), an immunosuppressive molecule capable of dephosphorylating AMP to adenosine, is intricately implicated in both adaptive and innate immune responses. However, it is unknown whether CD73 can influence the transition of macrophages and microglia between the M1 and M2 phenotypes [Lu F. et al., 2018].

Ecto-5’-nucleotidase (CD73) is a glycosylated protein with a molecular weight of 70 kDa that is found on the outer layer of the plasma membrane and catalyzes the breakdown of extracellular AMP into adenosine and phosphate. A significant mouse cerebral 5’-nucleotidase called CD73 mediates about 85–95% of the murine AMP-hydrolyzing abilities. CD73 is hypothesized to be intricately linked to immune- and inflammatory-related brain developmental disorders via regulating extracellular adenosine levels. Its impact on secondary spinal cord injury, however, is still unclear [Lu F. et al., 2018]. In order to catalyze the conversion of AMP into phosphate and adenosine, CD73, a glycosylphosphatidylinositol (GPI) anchored cell surface protein, plays a crucial function in adenosine signaling. The proliferation, migration, invasion, and medication resistance of different cancer cells have all been connected to this mechanism. However, there is little literature on CD73's impact in SCI. According to Baud et al., CD73 is widely distributed in gliocytes and neurons. According to Braun et al1997 .'s report, focal cerebral ischemia increased CD73 glial expression. Additionally, Petrovic-Djergovic et al. showed that CD73 might control leukocyte trafficking to lessen infarcted region in an ischemic brain. Hou et al. found that the presence of CD73 deficiency

significantly increased pro-inflammation cytokine levels in the bilateral common carotid artery stenosis model of cerebral hypoperfusion. Together, these investigations offer significant new understandings of CD73's CNS neuroprotective effects [Lu F. et al., 2018].

4. CD73 and autoimmune encephalomyelitis

In the purine catabolic pathway, CD73 functions as a cell surface enzyme that catalyzes the conversion of AMP to adenosine. We hypothesized that CD73 mice would develop severe experimental autoimmune encephalomyelitis (EAE), an animal model for the central nervous system (CNS) inflammatory illness, multiple sclerosis, due to the potent immunosuppressive and anti-inflammatory effects of adenosine. Unexpectedly, CD73 mice were immune to EAE. But when given to naive CD73 T cell-deficient recipients, CD4 T cells from CD73 mice produced more proinflammatory cytokines than wild-type (WT) animals and were able to cause EAE. Therefore, a lack of T cell reactivity was not the reason for the protection from EAE seen in CD73 mice. Immunohistochemistry revealed that compared to WT mice, CD73 animals had less infiltrating lymphocytes in the CNS. Importantly, vulnerability to EAE was induced in CD73 mice following the transfer of WT CD73+CD4+ T cells, indicating that CD73 expression is required for the production of illness either on T cells or in the CNS. [Mills J.H. and others, 2008]

Immunohistochemistry revealed that CD73 expression was low on brain endothelial cells and high in the choroid plexus epithelium, which controls lymphocyte immunosurveillance between the blood and cerebrospinal fluid, in the search for the source of CD73 in the CNS that might facilitate lymphocyte migration. We draw the conclusion that CD73 expression and adenosine receptor signaling are necessary for the effective entry of lymphocytes into the CNS during EAE development because blocking adenosine receptor signaling with the A2a adenosine receptor-specific antagonist SCH58261 protected WT mice from EAE induction [Mills J.H. et al, 2008]. In order to counterbalance ATP-mediated immune stimulation, adenosine works as a negative feedback signal, which reduces the collateral damage to healthy tissues and prevents unchecked inflammation. Additionally, it has recently been proposed that the immunosuppressive properties of regulatory T cells are mediated through CD73's production of adenosine (T-regs). Adenosine and CD73 have a more significant impact on CNS lymphocyte infiltration during EAE than they do on the regulation of neuroinflammation [Mills J.H. et al., 2008].

Knowing where CD73 must be expressed for T cell migration into the CNS is crucial. Several epithelial and endothelial cells, as well as some T cell subsets, express CD73. There is evidence that this A1-A2a relationship, where A1 signaling is protective and A2a signaling increases inflammation, is significant in mediating neuroinflammation in the CNS. An essential part in controlling inflammation in the CNS appears to be played by CD73-generated adenosine signaling at the choroid plexus. [Mills J.H. and others, 2008]

5. CD73 and Multiple Sclerosis

The central nervous system (CNS) is impacted by the chronic, disabling inflammation of multiple sclerosis (MS). Multiple regions of the brain and spinal cord are affected by immune-mediated axonal demyelination in MS patients, which results in a progressive loss of neurological function. Despite years of study, the cause of MS is still a mystery. Numerous studies show that the immune system is crucial to MS progression, despite the presence of genetic and environmental risk factors. Experimental autoimmune encephalomyelitis (EAE), the dominant MS animal model, has been used in research to show, for instance, how myelin antigen-specific CD4+ T lymphocytes can cause CNS inflammation, demyelination, and neurodegeneration, which can lead to the loss of motor function (paralysis) [Mills J.H. and others, 2008]

Autoreactive immune cells must enter the CNS for MS or EAE to develop. The blood-brain barrier (BBB), which is made up of specialized endothelial cell tight junctions, and other structural features of the brain typically regulate the amount of lymphocytes that can enter the central nervous system (CNS). Under physiologically normal circumstances, the brain is susceptible to immunosurveillance, but in MS and EAE, disease development is frequently correlated with greater lymphocyte infiltration/extravasation. Experimental MS treatments that aim to either prevent lymphocyte trafficking to the CNS or reduce inflammation have shown different degrees of success in human studies in recent years. We investigated whether CD73 (ecto-5'-nucleotidase), a cell surface enzyme that catalyzes the formation of extracellular adenosine, has a role in the development of EAE disease because adenosine has been shown to regulate leukocyte migration across endothelial barriers and the production of inflammatory cytokines. [Mills J.H. and others, 2008]

Treatment with IFN lowers the relapse rate in MS, however the drug's precise mechanism of action is yet unknown. Ectoenzyme CD73, also known as ecto-5' nucleotidase, dephosphorylates adenosine monophosphate (AMP) precursor to generate adenosine. Adenosine, the byproduct of CD73, is known to have both anti-inflammatory and neuroprotective effects, and it is also known to be abundantly present at sites of inflammation [AIRAS L. et al., 2007]. Our early research has demonstrated that systemic administration of IFN to MS patients in vivo increases the production of ecto-5' nucleotidase on endothelial cells (ECs) both in vitro and after. The majority of MS patients also had an increase in soluble serum CD73 levels after receiving IFN therapy. This was significant since it correlated with the clinical result. A confirmation of CD73 expression on the microvasculature of the central nervous system (CNS) was made using frozen tissue sections from MS brain tissues collected after autopsy. The positive effects of IFN on MS may be facilitated by adenosine, a known neuroprotective drug [AIRAS L. et al., 2007].

Data about NT5E expression in MS patients are scant despite the known significance for immune response imbalance in the etiology of multiple sclerosis (MS). Studies have evaluated the expression of NT5E in the peripheral blood of MS patients and healthy individuals to elucidate its function in the etiology of MS. The Multilevel Bayesian model's findings revealed no discernible difference in the expression of NT5E between all MS patients and control persons. When compared to male controls, its expression was noticeably reduced in male MS patients. In any research subgroup, there was no discernible relationship between age and NT5E expression. Surprisingly, NT5E transcript levels demonstrated a sensitivity of 92.31 percent and a specificity of 80 percent for MS disease detection. Based on AUC values, NT5E transcripts have an 86.2 percent diagnostic power. Particularly in MS patients who are men, the expression level of this gene may be employed as a possible marker [Ghafouri-Fard S. et al., 2019].

2. Peripheral nervous system: ROLE of CD73 IN OPHTHALMOLOGY

1. Retinal Photoreceptor Precursor Cells

The use of stem cells in ophthalmological disorders that affect both the anterior and posterior segments is examined in this chapter. The clinical trials that have made the most contributions to identifying the function and promise of stem cell regeneration treatment in corneal and retinal disease are reviewed by the authors. Without ignoring any potential negative consequences of using this therapy, the outcomes reported in the scientific literature are examined and discussed. The most work was put towards researching the potential applications of limbal epithelial stem cells in the anterior section (LESCs). They were the first stem cells to be identified at the anterior segment level and are currently the only ones being used in therapeutic settings with

positive outcomes. As of right now, corneal scarring and corneal stem cell shortage have been successfully treated [Vingolo E.M. et al, 2020]. Stem cell therapy may be used to treat age-related macular degeneration, Stargardt's disease, and retinitis pigmentosa, among other degenerative retinal illnesses. The use of cell therapies—in particular those that employ ADSC—is then discussed in terms of how they can help slow the progression of retinal degenerative illnesses through a variety of means. They can be categorized as follows: neurotrophism, oxidation, vascular alterations, apoptosis, inflammation, or immunology. These mechanisms encompass a variety of biological features. Later on, it is discussed how cell grafts can be used in ophthalmology as well as the best strategy for cellular surgery. The author's method and potential outcomes in the development of degenerative retinopathy are detailed in detail [Vingolo E.M. et al, 2020].

Adult tissues that experience spontaneous synaptic turnover include the mature retina and olfactory bulbs. Schoen and Kreutzberg have demonstrated that production of 5'nucleotidases at synaptic connections in adult rats contributes to this lasting synaptic alteration in the olfactory bulbs. Olfaction plays a crucial role in animals' typical environmental adaption, social behavior, and capacity for social self-determination. Additionally, olfactory deficiency is linked to neurological and neuropsychiatric conditions like Alzheimer's, Parkinson's, and Huntington's illnesses. According to Kulesskaya N. et al. (2013), there is no proof that CD73-deficient animals have olfactory system defects.

Around the time of birth, mouse retinal subpopulations that expressed CD73 first emerged, and their numbers thereafter rose sharply, eventually accounting for more than 90% of the adult retinal cells. At the first postnatal day, the majority of CD73+ cells were postmitotic and rhodopsin-negative. However, the majority of these cells only expressed rhodopsin and not s-opsin in the mature retina. The concept that CD73 is an early photoreceptor lineage marker is supported by the fact that CD73+ cells developed into rhodopsin-positive cells more quickly in reaggregation cultures than CD73 cells did. The effects of Nrl and Crx, two transcription factors known to be expressed in the photoreceptor lineage, on retinal cells show that CD73 is genetically downstream of Crx in the lineage that leads to the development of rod cells. Rhodopsin and CD73 expression patterns were also found to be correlated in the adult retina of the common marmoset monkey [Koso H. et al, 2009].

A cell surface marker for mature rod cells and cone/rod common progenitors in mice, CD73 is genetically located between Nrl and Crx. In order to purify photoreceptor cells for transplantation targeted at the regeneration of photoreceptors, CD73 can be a beneficial tool. The expression of CD73 was conserved in monkey rod cells [Koso H. et al., 2009]. Six different types of neurons and one type of glial cell make up the laminar structure that makes up the neural retina of vertebrates. Photoreceptors make up the outer nuclear layer (ONL), and the particular loss of these cells results in a number of serious retinal disorders, including retinitis pigmentosa. 1,2 A lot of work is being put into understanding the processes involved in the regeneration of photoreceptor cells, which is a crucial stage in the recovery of vision. One method for regenerating the neural retina through transplantation is the isolation of retinal progenitor cells or progenitors of the photoreceptor lineage. 3 However, due in part to a paucity of markers that may be used to distinguish the various phases and lineages of retinal cells, these cell groups have not yet been sufficiently defined. These substances are intracellular, which restricts their usefulness for cell enrichment even though the patterns of expression of transcriptional factors thought to be involved in retinal development may represent the developmental stage. Determining surface markers that can be utilized to identify particular retinal cell subpopulations is crucial. Surface antigens make it possible to isolate a certain fraction of cells from a cell mixture without causing any harm to the cells, which makes it easier to characterize cell lineages and find the variables that control cell proliferation and differentiation. [Koso H. and Others, 2009]

As the retina develops, the expression of the CD73 antigen rises. The 70-kDa glycosylphosphatidylinositol (GPI)-anchored cell surface protein CD73, also known as ecto-5’-nucleotidase, catalyzes the extracellular conversion of 5’-adenosine monophosphate to adenosine. A hallmark of the photoreceptor lineage's early stages is CD73. It is believed that CD73 is located genetically after Crx. [Koso H. and Others, 2009]

Both groups of immature and precursor photoreceptor cells are marked by CD73. Regarding transplantation applications for the treatment of retinal disorders, this is significant. It was demonstrated that the isolation of CD73 is an effective method for achieving photoreceptor cell production by transplantation using reaggregation cultures of retinal cells. [Koso H. and Others, 2009]

2. *Retinal inflammation and adenosine*

A1R, A2AR, A2BR, and A3R are the four subtypes of adenosine, an endogenous purine nucleoside that is widely dispersed throughout the body and interacts with G protein-coupled receptors. Adenosine has a wide range of uses, but it is increasingly understood to be a crucial immune response mediator. Chronic neurodegenerative disorders are characterized by neuroinflammation, which also plays a role in the pathogenesis of various retinal degenerative diseases. The regulatory functions of adenosine receptors in the onset and progression of retinal illnesses are being clarified using animal models of those conditions. There is growing evidence that diseased circumstances change the retina's adenosinergic system, impairing retinal functioning. The focus of this review is on the functions of adenosine and the adenosinergic system components (receptors, enzymes, and transporters) in the neuroinflammatory processes that occur in the retina. The development of novel therapeutic strategies will be facilitated by a deeper comprehension of the molecular and cellular mechanisms of the signaling pathways regulated by adenosine that underlie the start and progression of retinal disorders. In 2020, Santiago A. R. et al.

Age-related illnesses of the central nervous system (CNS), such as retinal degenerative diseases, frequently exhibit chronic inflammation (Madeira et al, 2015). An extremely well-organized tissue with both neural and non-neuronal cells makes up the retina. The retinal pigment epithelium (RPE) and the inner limiting membrane (ILM), a foundation membrane made of extracellular matrix proteins that delineates the boundary between the retina and the vitreous humor, both serve to limit the retinal tissue. In 2020, Santiago A. R. et al.

There are studies focusing on the functions of the cells engaged in the immune response of the retina, taking into account the involvement of neuroinflammation in the pathogenesis of retinal degenerative diseases. We review the role of adenosine and the adenosinergic system in the inflammatory response in the retina, highlighting potential therapeutic approaches to control retinal inflammation because adenosine is a neuromodulator of the CNS that is known to be involved in inflammatory processes [Santiago A. R. et al, 2020]

Immune response's main participants are:

- ☐ Microglia, important participants in the immunological response
- ☐ Müller cells and astrocytes: the immune response's role for macroglia
- ☐ The retinal pigment epithelium regulates immunity
- ☐ Signaling and Adenosine Receptors

The resident immune cells in the CNS are known as microglial cells; they were initially identified as a distinct cell type, physically different from other glial and neuronal cells, by Pio del Rio-Hortega in 1932 [Santiago A. R. et al, 2020]. Adenosine is a purine nucleoside that occurs naturally and is widely dispersed throughout the body. It is involved in a number of fundamental processes, including the creation of purinergic nucleic acid base, the metabolism of amino acids, and the control of cellular metabolic state (Trincavelli, Daniele, & Martini, 2010). Adenosine functions in the central nervous system (CNS) as a neuromodulator and homeostatic regulator, regulating synaptic activity, neurotransmitter release, and neuronal excitability (Chen, Lee, & Chern, 2014).

Inflammation is one of the physiological and pathological processes that adenosine is involved in [Santiago A. R. et al, 2020]. It also functions as an intracellular messenger.

Different G-proteins are coupled by adenosine receptors to activate various intracellular signaling pathways. Adenylate cyclase (AC) regulates the levels of cyclic AMP (cAMP) by differential coupling of the adenosine receptors. The activation of the A1R and A3R, which are linked to the Gi/o proteins, prevents the synthesis of cAMP. On the other hand, the activation of A2AR and A2BR leads to an increase in cAMP synthesis since these receptors are connected to Gs/olf proteins. The adenosine receptors are classified as facilitatory or inhibitory receptors, respectively, depending on their capacity to stimulate or inhibit the AC [Santiago A. R. et al, 2020]. Pathological situations affect the retina's other adenosine metabolism-related enzymes. According to Ahmad et al. (2014), adenosine kinase levels are higher in the retina of an animal model of traumatic optic neuropathy than they are in the retina of type 1 diabetic animals, suggesting that changes in the components of the adenosinergic system may vary depending on the pathological condition. [Santiago A. R. et al., 2020]

Inflammation is one of the biological processes that adenosine plays a role in. Controlling neuroinflammation may be a viable therapeutic strategy since chronic neuroinflammation plays a significant role in many retinal disorders and contributes to degeneration. The adenosinergic system has been linked to the control of neuroinflammation in the retina by a significant quantity of evidence, making it a viable target for therapeutic intervention [Santiago A. R. et al, 2020].

10. Functions in the Integumentary system:

1. ROLE of CD73 IN DERMATOLOGY

In connection to the immunogenicity of the melanoma tumor and the characteristics of the inflammatory milieu that support immune suppression throughout the course of the illness, the mechanisms of immunological escape have been extensively studied. These discoveries have lately provided advantages for immunotherapy-based strategies as justification for defeating the immune escape. However, due to the immunosuppression brought on by the tumor environment, additional mechanisms, such as the adenosine generated by ectonucleotidase CD73, also play a role in the evolution of melanoma. However, CD73 has lately come to light as a viable therapeutic target as well as a poor predictive biomarker. The CD39/CD73/adenosine pathway in melanoma activates the main immune escape mechanisms, and prospective treatment approaches center on the management of CD73 downstream adenosine receptor signaling. These data serve as the foundation for immunological combination translational techniques, and CD73 could be used as a predictive biomarker in metastatic melanoma. 2019 [Passarelli A. et al]

Despite immunotherapy's unquestionable improvements in progression-free survival (PFS) and overall survival (OS), the incidence of cutaneous melanoma has grown globally during the past ten years. However, due to the lack of biomarkers to choose potential responders and to quickly identify resistant patients whose molecular pathways have only been partially uncovered, immune checkpoint inhibitors only assist a small subset of patients. 2019 [Passarelli A. et al]
Melanoma also exhibits significant variation for CD73 expression. The presence of activating MAPK mutations, mitogenic and inflammatory signals, necrosis, and sample type (primary, metastatic, or relapse tissue) all affect CD73 expression in melanoma [Harvey J.B., 2020].

It is interesting that effector T cells require an anabolic environment, whereas naive T-lymphocytes primarily employ the available resources, including fatty acids and glucose, to enhance energy generation by oxidative phosphorylation. Furthermore, T cells attracted to peripheral regions become activated after co-stimulatory

molecules bind to and stimulate the T-cell receptor (TCR), reprogramming naive T cells' metabolism to an anabolic state that supports the proliferation leading to the effector functions. 2019 [Passarelli A. et al]

However, the adenosine receptors differently activate these inhibitory effects. The most prevalent subtype of the A2A receptor (A2AR), which triggers inhibitory signals to control the activity of T cells, NK cells, NK-T cells, neutrophils, macrophages, and dendritic cells, is in fact activated in both innate and adaptive immunity (DCs). Through the activation of A2AR, CD73 reduces the cytotoxicity, proliferation, cytokine generation, and efficiency of antigen processing and presentation stimulated by these cell types [Passarelli A. et al., 2019].

Therefore, the formation of the so-called "purinergic milieu," which surrounds immune system cells, and the regulation of NAD + nucleosidase and adenosine in the cancer microenvironment depend crucially on either the CD38/CD203/CD73 or the CD39/CD73 pathways. 2019 [Passarelli A. et al]

The CD39/CD73/adenosine axis, which causes the immune system to malfunction, is likely relevant for melanomagenesis in addition to ischemia, hypoxia, inflammation, and trauma. In fact, there are various ways to accomplish the immunosuppression caused by adenosine on immune effector cells in the melanoma microenvironment. First, adenosine can dephosphorylate SHP-2 or STAT-5 to interfere with signals sent by the IL-2 receptor and limit the production of cytokines [Passarelli A. et al., 2019]. Adenosine-dependent mechanisms have also been shown to be used by melanoma cells to directly limit the proliferation of naive CD4 + T cells and, to a lesser extent, CD8 + cells. This effect was quickly reversed with the use of certain CD73/CD38 inhibitors. As a result, it has been proposed that adenosine is a critical factor in the melanoma cell's ability to evade adaptive immune regulation [Passarelli A. et al., 2019]. Additionally, both immunological and melanoma cells have adenosine receptors, and the precise activation of A1R controls their chemotaxis and motility. A2R and A3R, on the other hand, control angiogenesis, metastasis, and the growth of melanoma cells. Recent research on the function of CD73 in the development of melanomas found that activating the MAPK pathway encourages melanoma cells to overexpress CD73, whereas inhibiting BRAF/MEK signaling inhibits this overexpression [Passarelli A. et al., 2019].

Additionally, CD73 supports the flexibility of melanoma cells and aids in the transition from a proliferative to an invasive phenotype in these cells. In particular, the low expression of melanocyte lineage transcription factor (MITF), the up-regulation of CD73, and the activation of putative genes mediating the epithelial-to-mesenchymal transition (EMT) are characteristics of the invasive profile [Passarelli A. et al., 2019].

Since a sizeable number of metastatic melanoma patients treated with checkpoint inhibitors do not experience a therapeutic benefit or develop resistance, prognostic and predictive biomarkers are urgently needed in the era of immunotherapy [Passarelli A. et al., 2019].

The measurement of soluble CD73 for predictive purposes has been suggested by a distinctive connection between levels of soluble CD73 and outcome in patients with metastatic melanoma treated with anti-PD1 checkpoint drugs. This finding helps to define the prognostic significance of CD73 in melanoma. Since high basal levels of soluble CD73 have been linked to the poorest response to immunotherapy, CD73 is specifically advocated as the strongest predictive factor for OS and PFS [Passarelli A. et al., 2019].

Due to CD73's immunosuppressive role in the tumor microenvironment, it is now becoming clear how it affects melanoma growth and progression. This presents a possible possibility for the development of targeted therapies. Monoclonal antibodies directed against CD73 or chemical inhibitors of adenosine receptors, both of which have comparable efficacy in restricting melanoma cell growth, have recently been proposed in mouse melanoma models. [Passarelli A. et al., 2019]

A new cell surface indicator of CD25highFoxp3+ regulatory T cells is CD73 (T-regs). In addition to differentiating T-regs from other cell types, concordant expression of these two ectoenzymes also produces pericellular adenosine, which has been shown to inhibit the proliferation of activated T effector (Teff) cells. We looked at the frequencies and phenotypes of CD73-expressing T-regs and related receptor adenosine receptor 2A (A2A R) in peripheral blood of patients with various types of psoriasis because it is currently unknown whether human ectoenzyme (CD73) is responsible for the impaired suppressive activity of T-regs in psoriasis [Huang Q. et al., 2018].
Only a small percentage of human T-reg were found to display CD73 on their surface, which has been identified as a distinctive surface identifier of murine T-reg (almost 13 percent). Given that CD73 and CD39 work synergistically, it is possible that CD73 is easily lost from the surface of human lymphocytes based on the imbalanced expression of these two molecules [Huang Q. et al, 2018].

Three different kinds of psoriasis were evaluated for peripheral blood T-reg CD73 expression levels. 2018 [Huang Q. et al]. Immune checkpoint inhibitors that block the PD-1 (programmed cell death protein 1) receptor on T cells have produced outstanding therapeutic results in metastatic melanoma. But the majority of people are resistant to treatment. Through the breakdown of AMP by CD73, extracellular adenosine is produced, which inhibits T-cell-mediated defenses against cancer. The expression and activity of soluble CD73 in the sera of melanoma patients receiving anti-PD-1 cytotoxic T-lymphocyte-associated antigen 4 treatment were examined in this study. In 2020, [Turiello R, et al]

A deeper comprehension of the processes that control antitumor immunity and affect the therapeutic efficacy of immune checkpoint inhibitors is required. It is also necessary to identify predictive biomarkers to inform immunotherapeutic decisions and, more critically, prospective new co-targets to boost the effectiveness of anti-PD-1 therapy [Turiello R, et al, 2020].

Both the membrane-bound and soluble forms of the ecto-nucleotidase CD73 are capable of hydrolyzing extracellular AMP in a rate-limiting way to produce extracellular adenosine. The administration of immune checkpoint inhibitors in conjunction with an anti-CD73 antibody improved antitumor immune response and efficacy in mouse models of melanoma, breast cancer, colon cancer, and prostate cancer. Monoclonal antibodies against CD73, which are currently being studied in clinical trials in combination with anti-PD-1 drugs in cancer patients, are the latest generation of immune checkpoint inhibitors. Additionally, new research indicates that overexpression of CD73 is frequently linked to poor survival in human tumors or in the peripheral blood of cancer patients, suggesting that CD73 may also function as a predictive biomarker in cancer. In 2020, [Turiello R, et al]

In patients undergoing anti-PD-1 therapy, elevated baseline CD73 activity is highly related with a lower response and survival [Turiello R, et al, 2020]. In many cancer types, the soluble form of CD73 in peripheral blood has been studied. Previous research on head and neck cancer showed that the serum expression of CD73 was higher in cancer patients compared to healthy individuals, and its enzymatic activity was related to the stage of the disease. Patients with colorectal cancer, prostate cancer, and cervical cancer all had elevated blood levels of soluble CD73 [Turiello R, et al, 2020]. High levels of soluble CD73 expression are linked to a reduced survival, according to recently released data from patients with colorectal cancer liver metastases. These findings suggest that soluble CD73 may function as a non-invasive blood-based cancer outcome indicator. However, it is not apparent whether soluble CD73 is a clinically effective biomarker for melanoma patients' prognosis and treatment response.

Increased CD73 enzymatic activity in the peripheral blood may likely be a reflection of increased tissue CD73 expression. We were unable to examine the tumor's CD73 level in this retrospective analysis, though. Therefore, we are unsure if there is a relationship between the levels of soluble CD73 found in the blood and those that are expressed in tumors [Turiello R, et al, 2020].

When anti-PD-1 medicines were used alone as the first line of treatment for patients, baseline CD73 activity was found to be strongly linked with response to these drugs. In addition, PFS was substantially correlated with serum CD73 activity in this final group of patients receiving first-line treatment. These findings may point to the potential value of CD73 as a biomarker of response in patients receiving first-line anti-PD-1 therapy. High-soluble CD73 activity might be a sign of immunotherapy resistance [Turiello R, et al., 2020]. Extracellular adenosine, which is produced mostly by the enzyme CD73 and is known to be a powerful inhibitor of the T-cell-mediated antitumor immune response, may work against the antitumor immunity, limiting the effectiveness of anti-PD-1 medications. This CD73-mediated mechanism of resistance is expected to be significant, particularly in patients treated first with anti-PD-1 drugs, as opposed to patients who receive these drugs in a second line of treatment after progressing on a first line of therapy. Finally, we saw that, significantly, the CD73 activity did not alter from the baseline level following the 3 months of treatment that were available for this investigation. In 2020, [Turiello R, et al]

CHAPTER III: CD73 clinical benefits and limitations

1. Signaling pathway and disease expression

1. Valuation of CD73 expression

Leukemia, malignant glioma, melanoma, ovarian, colon, breast, and bladder cancer are only a few of the cancers that express CD73, which also promotes tumor growth, metastasis, and medication resistance [Zhang B. et al, 2019]. In order to accurately recreate the complex physiology and dynamic cellular interactions during pathogenesis, in vivo models are required because vascular disease is complex and the various manifestations are influenced by variations in vascular bed architecture, exposure to shear and mechanical forces, cell types involved, and inflammatory responses. Murine knockout models are frequently employed by researchers to examine the function of a particular gene or pathway in complex disease features [Joolharzadeh P. et al., 2019]. These models, while useful, are not without flaws, and this is especially true in the case of CD73 (cluster of differentiation 73), the extracellular enzyme that converts AMP into adenosine. While CD73-deficient people exhibit the complex phenotype of arterial calcification, arteriomegaly and tortuosity, and calcification in tiny joints, CD73-deficient mice do not exhibit an overt phenotype at baseline. The distinctions between mouse and human systems are highlighted in this review, along with the possibility of using discoveries from mice to shed light on human situations [Joolharzadeh P. et al, 2019].

In ACDC patients, vascular calcification is the most notable phenotype. Numerous cardiovascular disease states are linked to ectopic calcification in the vasculature, which also has a strong correlation with increased cardiovascular risk. Prior to the discovery of small calcifications in early-stage plaques and, intriguingly, in the medial layer of otherwise healthy adults by more advanced imaging techniques, it was initially found in advanced atherosclerotic plaques and thought to be an uncontrolled and nonbiological consequence of degeneration. Vascular calcification is used as a risk-predicting biomarker since it is now understood that both atherosclerotic (intimal) and vessel wall (medial) calcification are active biological processes that change vascular homeostasis and increase disease pathologies. Pathological calcification cannot be stopped or reversed clinically at the moment. [Joolharzadeh P. et al, 2019]

According to scientific data, the immunosuppressants dexamethasone and methotrexate function by stimulating the release of adenine nucleotides and raising CD73 expression, respectively. Although not rheumatoid-like, the absence of local CD73-mediated adenosine signaling is hypothesized to be the cause of the periarticular calcification seen in the joint capsules of ACDC patients. In fact, the mechanism by which methotrexate is effective in the treatment of rheumatoid arthritis is by increasing levels of adenosine. et al. [Joolharzadeh P., 2019]. There is a drive to include CD73 deficiency in the spectrum of rheumatologic diseases due to early-onset joint discomfort. The presence of CD73, which is protective in a well-established murine model of rheumatoid arthritis, and evidence of low CD73 expression on synovial lymphocytes in more severe forms of juvenile idiopathic arthritis both lend support to this idea that calcification and inflammatory processes interact in this particular local environment. Anti-inflammatories do not relieve the joint pain in ACDC, though, and there is no sign of an autoimmune condition. According to more research, the adenosine signaling deficiency caused by A3AR ablation causes articular cartilage to deteriorate as a result of chondrocyte activity in the aggrecanase and collagenase enzymes. The fact that adenosine signaling is also antinociceptive raises the intriguing question of whether the joint-capsule phenotype of ACDC patients is immune driven or whether the absence of local

adenosine in these areas makes pain more sensitive without triggering an immune response [Joolharzadeh P. et al, 2019]. The field of cancer has done a lot of study on CD73. In light of the immunosuppressive properties of CD73-generated adenosine in mouse models, inhibiting CD73 activity is currently being investigated as a therapeutic target. The extracellular fluid of solid tumors was shown to contain immunosuppressive levels of adenosine, which conveyed its effects by activation of the A2aARs on T cells. Higher CD73 activity is linked to a worse cancer prognosis. Mice lacking CD73 are less susceptible to carcinogenesis and had slower tumor invasion and growth. When the tumor cells themselves were CD73-deficient, CD73-deficient animals in a melanoma model showed a reduction in tumor growth [Joolharzadeh P. et al, 2019]. Murine models of heart failure have also been linked to CD73-mediated inflammatory processes. Inflammation and cytokine production are increased in heart failure patients. Heart failure patients may benefit from the anti-inflammatory properties of T cells' CD73-generated adenosine. Heart failure is not known to occur in humans with CD73 loss; nonetheless, it will be interesting to watch for this in these individuals to see if disease burden and progression are worse [Joolharzadeh P. et al, 2019].

It will be fascinating to observe whether the rodent immunomodulating effects of CD73 blockage on cancer will be replicated in people given the variations between CD73 deficit in the vasculature of mice and humans. The calcific or tortuous phenotype seen in ACDC joints and blood vessels may also be caused by inflammation, as immune cells are found infiltrating calcified vessels [Joolharzadeh P. et al, 2019]. The ecto-5'-nucleotidase CD73, a recognized therapeutic target in cancer, plays a significant role in the synthesis of immune-suppressive adenosine in the tumor microenvironment. Extracellular ATP is used by the anticancer immune response to limit cell proliferation by activating T cells. However, two extracellular membrane-bound enzymes (CD39 and CD73) are overexpressed in the tumor micro-environment and efficiently hydrolyze ATP into AMP before further converting it into immune-suppressive adenosine [Chaloin L. et al., 2018].

A number of successful compounds discovered through virtual screening campaigns effectively inhibited recombinant CD73 with inhibition constants in the low micromolar range and demonstrated a non-competitive inhibition mode. The structure-activity relationships studies showed that a number of amino acid residues at the dimerization interface are involved in the tight binding of hit compounds and likely contributed to their inhibitory activity. Through the activation of T cells and subsequent release of pro-inflammatory cytokines, the immune response acts as a significant barrier for halting the spread of cancer. Extracellular ATP, which starts and controls this process by binding to P2X and P2Y receptors, affects a wide range of cells (including T and B lymphocytes, NK, macrophages, DC, neutrophils, and vascular endothelial cells), causing chronic inflammation and inhibiting regulatory cells. Although the concentration of extracellular ATP is very low in healthy tissues, it is abundantly released by dying cells and secretion in solid tumors, where its concentration can reach a few hundred micromolar [Chaloin L. et al., 2018]. ATP often serves as an alarm signal in the tumor microenvironment, permitting the recruitment of immune cells and assisting in the process of immunogenic cell death. The two ectonucleotidases work together, however, to swiftly and sequentially breakdown ATP into AMP and subsequently adenosine when high ATP concentrations are linked to high levels of CD73 expression on both immune and cancer cells. Due to adenosine binding to P1 receptors (mostly A2a and A2b) expressed on immune cells, an abnormally high adenosine concentration is created in the tumor microenvironment, which causes a powerful inhibition of the antitumor immune response [Chaloin L. et al., 2018].

Ecto-5'-nucleotidase is a non-covalently coupled homodimer that is expressed on endothelial, immune, and tumor cells. It is an anchoring cell surface protein. In addition to its membrane-attached form, CD73 also comes in a soluble and circulatory form with comparable enzymatic activity. It's interesting to note that this soluble form was also present in crude extracts from cells and organs, which was probably produced by phospholipase activity on the GPI-anchored precursor. Most B lymphocytes, T cells, including Th17, NK cells, and myeloid-derived suppressor cells in human peripheral blood express CD73. In addition, CD39 and CD73 can co-express in these cells [Chaloin L. et al., 2018]. Multiple types of solid tumors and endothelial cells have been reported to overexpress CD73 in the tumor microenvironment where hypoxia is predominating. The prognosis for individuals undergoing anticancer therapy is typically bad for this group of cancers, which includes colorectal, breast,

bladder, pancreatic, ovarian, leukemia, and melanoma. There are a select few cases that highlight CD73 as a useful prognostic indicator for the clinical investigation of endometrial and breast carcinomas. The endometrial epithelial barrier integrity in endometrial malignancies may have changed specifically as a result of this disparity between opposed tasks of CD73, or it might be a result of the soluble version of CD73 predominating in these tumors (sCD73). In fact, sCD73 plasma concentrations were shown to be higher in cancer patients or people with acute inflammatory pancreatitis than in healthy people. According to these findings, the blood level of sCD73 may be upregulated as a prognostic indicator of tissue inflammation and tumor hypoxia. The effect of adenosine binding to A1 and A3 receptors has also been demonstrated to increase cell proliferation, migration, invasion, and adhesion to the extracellular matrix in human breast cancer [Chaloin L. et al., 2018].

Additionally, CD73 deficiency in mice was investigated and linked to enhanced antitumor immunity or resistance to in vivo carcinoma development. The use of either monoclonal antibody inhibiting CD73 enzyme activity has demonstrated the involvement of CD73-produced adenosine in cancer growth and metastasis. As a result, ATP purinergic signaling could be used to reestablish the immunological response. All of these factors have led to the designation of CD73 as a prospective therapeutic target for the creation of fresh anticancer medications. ADP, ATP, and adenosine 5'-[, -methylene] diphosphate (a non-hydrolyzable ADP analog known as APCP), which all function as competitive inhibitors, were the first CD73 inhibitors to be described. Consequently, recently developed and investigated small molecule inhibitors derived from APCP have shown effective competitive inhibition of CD73 [Chaloin L. et al, 2018].

According to Wen and Kesari (2008), glioblastoma (GB) is the most prevalent and lethal primary malignant brain tumor due to its great proliferative potential and strong chemoresistance (Sarkaria et al., 2008). Additionally, the blood-brain barrier's (BBB) impermeability makes it difficult to distribute chemotherapy drugs for the treatment of GB (Neuwelt et al., 1982). Even with treatment, the median survival time for GB patients is currently 16 months (Topkan et al., 2018). It is one of the most fatal forms of brain cancer that may affect a person, and even with treatment, its prognosis is quite poor. Cell adhesion, proliferation, invasion, and angiogenesis are just a few of the biological processes that the extracellular adenosine-generating enzyme CD73 is engaged in, all of which can be hijacked by malignancies [Bynoe M. S. et al., 2019]. CD73 contributes to the pathogenesis of GB. Permeability glycoprotein (P-gp) and multidrug resistance-associated protein 1 expression were both decreased by the inhibition of A2B AR signaling (MRP1). Furthermore, temozolomide, a chemotherapy medication, potently accelerates GB cell death when A2B AR signaling is blocked. Together, these data imply that CD73 and A2B AR have a complex role in the development and progression of GB, and that treating GB patients by targeting the CD73-A2B AR axis can help them and provide new ideas for treatment [Bynoe M. S. et al., 2019]. The deadliest primary brain tumor, glioblastoma (GB), has a median survival time of about 16 months even with treatment. To increase patient survival and advance GB treatment, prophylaxes must be created. Adenosine produced by CD73 has been linked to cancer etiology, although its function in GB has not been clarified. Here, we showed that host CD73 plays a significant role in driving GB growth, its angiogenesis, and its invasiveness, as well as other aspects of glioblastoma pathogenesis. [M. S. Bynoe et al., 2019]

Tumor adhesion, invasion, proliferation, angiogenesis, and chemoresistance are all influenced by CD73 and adenosine (Wang et al., 2003). For instance, in hypoxic settings, activation of the transcription factor hypoxia inducible factor 1 (HIF-1) boosts CD73's production of extracellular adenosine (Adair, 2005). Vascular endothelial growth factor (VEGF) is released as a result of adenosine signaling through the A2A and A2B ARs, which promotes angiogenesis and endothelial cell proliferation to advance tumor growth (Adair, 2005) In 2019 [Bynoe M. S. et al]. Matrix metalloproteinases (MMPs), which are essential for angiogenesis and tumor invasion by destroying the extracellular matrix, are regulated by CD73 and AR signaling as well (Chan et al., 2006). (ECM). It has been demonstrated that MMP2 and MMP9 encourage GB angiogenesis and invasion and control vascular morphogenesis (Wang et al., 2003). However, it is still unclear how CD73 controls GB pathogenesis and whether host CD73 plays a role in tumor etiology. The pathogenesis of GB was largely influenced by both the host and GB-CD73. Through regulation of CD73 and/or the CD73-AR axis, our results offer a possible target for GB therapeutic intervention [Bynoe M. S. et al., 2019].

In the central nervous system (CNS), purinergic chemicals like adenosine play crucial functions in controlling cell survival and proliferation as well as mediating communication between glial cells and neurons. According to studies, adenosine significantly contributes to the emergence and spread of cancer by reducing the immune response (Antonioli et al., 2013). It remained unclear, though, how the host and GB-CD73 affected the etiology and development of GB. Host CD73 encourages the pathogenesis of GB, including making it more invasive. [M. S. Bynoe et al., 2019]

Furthermore, CD73 mice may exhibit considerably different host immunological responses to GB because to CD73's prominence as an immune regulator. It is crucial to understand that the host and GB may react to anti-CD73 antibodies or CD73 inhibitors in different ways, and that targeting particular cells may help to predict the treatment's result. [M. S. Bynoe et al., 2019]

Low uptake in healthy organs and tissues and tumor uptake depends on CD73 expression. As an imaging probe for the noninvasive assessment of CD73 expression levels in patients, radiolabeled 067-213 shows potential. Our findings call for additional clinical research to define the function of CD73 monitoring in patients undergoing immune therapies that target CD73 [Higashi T. et al., 2020]. Extracellular adenosine is a well-known immune suppressant; it has direct effects on antitumor effector cells as well as indirect effects on antigen-presenting cells, immunoregulatory cells including regulatory T-cells, and suppressor cells derived from myeloid cells. Adenosine is present in high concentrations in cancer tissues where it inhibits antitumor immune responses, in contrast to normal tissues where it is present in low concentrations. As a result, adenosine-mediated immunosuppression has received interest in the field of oncology and immunological responses associated with cancer. In 2020, Higashi T. et al.

The GPI-anchored ectonucleotidase CD73, whose activity controls the level of extracellular adenosine, dephosphorylates adenosine monophosphate to generate adenosine. CD73 is found on the cell surface membrane. Numerous tumor forms express the protein at high levels, and CD73 expression levels are associated with tumor development and patient survival. CD73 has been shown to be a possible biomarker for reactions to immuno treatment, radiation, and chemotherapy in a number of preclinical and clinical investigations. CD73 inhibition inhibits tumor growth and metastasis in animal models by triggering an immune response against the formation of malignancies. As a result, immune checkpoint treatment now targets CD73 as a promising target molecule [Higashi T. et al, 2020].

Noninvasive imaging can reveal the expression of therapeutic targets, both in cancer tissues and in healthy tissues, and it can forecast therapeutic outcomes and side effects of targeted medicines. Nuclear medicine imaging methods like PET and SPECT have excellent sensitivity and are measurable among the numerous molecular imaging approaches. There haven't been any reports, though, of noninvasive imaging that targets CD73 that can be used on clinical patients. Patient classification for CD73-targeted therapy would benefit from imaging with radiolabeled anti-CD73 antibodies. Extrapolation of preclinical findings to humans is required in order to take this promising probe to the clinic In 2020, Higashi T. et al.

Rich stroma in pancreatic cancer has a significant role in the disease's progression and resistance to treatment. Ryzhov et al. demonstrated that CD73-positive myeloid cells are immunosuppressive, proangiogenic, and tumor-promoting using pancreatic cancer cells, including MIAPaCa-2 cells. In contrast to cancers without lymph node metastasis, Messaoudi et al. showed that tumors with lymph node metastasis had greater levels of CD73 expression on cancer cells. All things considered, these studies point to the possibility that CD73-mediated adenosine signaling, which is essential for cell adhesion and encourages the localization of E-cadherin and -catenin to the cell membrane, may modify -catenin/WNT signaling, which in turn can reduce chemokine production from tumor cells and prevent T-cell infiltration. It's possible that noninvasive imaging of CD73 in MIAPaCa-2 tumor models will reveal new information on CD73's function in the pancreatic cancer immune milieu [Higashi T. et al., 2020].

1. *Effects of CD73 activity by tumor cells*

The expression of CD73 on tumor cells is modulated by a variety of circumstances. Breast cancer CD73 expression and its capacity to produce adenosine have been demonstrated to be adversely and predominately regulated by estrogen receptors (ERs). Therefore, in a subgroup of ER-negative tumor cells, the elevated expression of CD73 may be related to the development of breast cancer. Given that HIF-1 directly regulates CD73 gene transcription, CD73 expression is frequently increased in the tumor microenvironment when hypoxia is present. Additionally, a number of proinflammatory substances, such as prostaglandin E2, TGF-, IFNs, TNF, and IL-1, stimulate the production of CD73. Furthermore, both the Wnt and cAMP pathways are used to control CD73 expression in tumor cells. Since CD73 expression is suppressed in human melanoma cell lines by methylation-dependent transcriptional silencing, CD73 expression is also increased epigenetically. Melanomas that lack CD73 methylation in particular are more prone to relapse. In addition, melanoma cells express CD73 more readily when the MAPK pathway is active in conjunction with proinflammatory cytokines like TNF. A growing body of research demonstrates that a range of distinct cancer subtypes may exhibit abnormal CD73 regulation at the transcriptional and post-transcriptional levels (e.g., miRNA) [Zhang B. et al., 2019].

The activation and effector phases of the antitumor T cell response are adversely regulated by the extracellular adenosine produced by tumor cells that express CD73, and T cell death is also promoted. In addition, CD73 is necessary for cancer cell proliferation unrelated to immune control. On the other hand, breast cancer cells (MCF-7) that overexpress CD73 have higher cell viability and support the development of the cell cycle. Together, the adenosine produced by CD73 and its enzyme activity may encourage the proliferation of cancer cells. The pro-apoptotic chemicals in adenosine cause ovarian cancer cells and gastric carcinoma cells to undergo apoptosis, proving that this impact is not universal. In mouse models, the expression of CD73 on tumor cells also facilitates tumor spread, most likely due to the autocrine activation of A2BR. Chemotaxis and invasiveness are encouraged by the expression of CD73 on tumor cells or by the activation of additional adenosine receptors. Surprisingly, in a mouse breast cancer model, CD73 activity by tumor cells promotes VEGF production, which contributes to tumor angiogenesis. The druggability of CD73 in the context of cancer stem cell/cancer-initiating cell-directed therapy is highlighted by the fact that CD73 is also overexpressed on cancer stem cells or cancer-initiating cells and that CD73 inhibition attenuates sphere formation and tumor initiation. These findings call for additional in vivo research as they point to a complicated and contextual role for CD73 in controlling cancer cell survival, stemness, and immune suppression [Zhang B. et al, 2019].

2. *Effects of CD73 activity by nontumor cells*

Facts supported by scientific evidence include the following:

- ☐ The modulation of tumor immunity by CD73-mediated adenosinergic impact on a number of immune cell populations, including CD4, CD8, NK, MDSC, macrophage, B cell, and ILC2, has been thoroughly studied.
- ☐ Both tumor and endothelial cell CD73 synergistically contribute to tumor angiogenesis.
- ☐ Similar to the immunosuppressive impact of tumor cell CD73, CAF CD73 expression suppresses T cell-mediated antitumor immunity [Zhang B. et al, 2019].

vii. Endothelial cells

Within the tumor microenvironment, a subgroup of endothelial cells that express CD73 may aid in the angiogenesis process. In fact, when pulmonary microvascular endothelial cells were in vitro cultivated with cancer cell-conditioned media, WT mice generated more capillary-like structures in pulmonary microvascular

endothelial cells than CD73 hosts. When compared to CD73-deficient mice in vivo, tumor angiogenesis in WT mice was more extensive and dense. Furthermore, in various murine tumor models, therapy with anti-CD73 monoclonal antibody (mAb) or APCP resulted in impaired angiogenesis and reduced tumor growth. There was also proof that CD73 expression, but not the related enzyme activity, affects the in vitro development of capillary-like tubes by human umbilical vein endothelial cells (i.e., extracellular adenosine). Additionally, CD73 on tumor cells facilitates metastasis by adhering to endothelium without adenosine. When examined collectively, recent findings show that tumor and endothelial cell CD73 work together to promote tumor angiogenesis [Zhang B. et al, 2019].

After receiving intravenous injections of TRAMP-C1 prostate cancer cells or B16F10 melanoma cells, CD73 animals in an experimental lung metastasis model were discovered to be immune to tumor metastasis. It is noteworthy that host CD73's pro-metastatic actions were reliant on the expression of nonhematopoietic cells, most likely endothelial cells. On the other hand, tumor growth appears to be accelerated and limited tumor T cell infiltration is connected with endothelial cell CD73 expression. The majority of the available data suggests that CD73-expressing endothelium contributes to the growth and spread of tumors [Zhang B. et al, 2019].

viii. T cells

By stifling the immune response, regulatory T cells (T-regs; CD4+CD25+FoxP3+) facilitate immunotolerance and aid tumor cells in evading immunosurveillance. The extracellular adenosine produced by CD73 is necessary for one of the primary mechanisms of T-reg-mediated tumor immunosuppression. T-regs frequently co-express CD39 and CD73, which CD73 expresses abundantly. T-regs produce an enzymatically driven buildup of immunosuppressive adenosine when CD73 and CD39 are together. As a result, T-regs produced from CD73 or CD39 mice are less effective at suppressing the immune system. CD73 T-regs do not aid in the formation of tumors, in contrast to WT murine T-regs. Similar to this, in vitro suppression of human T-reg CD73 reduces their immunosuppressive capacity. Coincidentally, circulating T-regs isolated from patients with head and neck cancer express more CD73 than do healthy donors. Natural human T-regs are frequently CD39-positive, in contrast to murine T-regs, but they seldom or never express cell surface CD73. Patients with melanoma see a substantial increase in inducible CD39+CD73+ T-regs in their peripheral blood, sentinel lymph nodes, and tumor-infiltrating lymphocytes, particularly in response to high-dose IL-2 therapy. It is generally acknowledged that human T-regs produce adenosine through paracrine interactions with nearby CD73-expressing cells and/or tumor-derived exosomes, even if the precise function of CD73 on human T-regs is still being fully described.

Murine CD4+Foxp3 conventional T cells and CD8+ T cells express CD73 when exposed to TGF-, in addition to T-regs. Human CD8+ T cells in the periphery of healthy donors express CD73, and CD8+ T cell activation reduces CD73 expression. Memory CD8+ T cells in tumors show high levels of CD73, compared to low levels on effector CD8+ T cells that have undergone terminal differentiation. A subset of CD4+ effector T cells that are enriched in polyfunctional Th1/17 cells are also linked to CD73 expression. Multicolor immunofluorescence supports the finding of CD73+CD4+ effector T cells and CD39+ T-regs within the immunological infiltrates in human breast and ovarian cancers that have undergone resection. In mouse tumor models, elevated CD73 levels are linked to enhanced adenosine synthesis, immunosuppressive Th17 actions, and tumor progression. Contrarily, genetically altering CD73 or programming Th17 cells ex vivo with IL-1 rather than TGF- enhances the anticancer effects of adoptive Th17 cell treatment and increases stemness [Zhang B. et al., 2019].

ix. Natural killer cells

Natural killer (NK) cells express CD73 at high quantities in the tumor microenvironment but at low levels in healthy persons. Through the production of adenosine, NK cells with CD73 expression have inhibitory properties. A2AR is mostly expressed by NK cells, and it is further elevated under pathological circumstances including lung

inflammation and damage. Activation of the A2AR inhibits the activation and cytotoxic activity of NK cells. As a result, NK cell antitumor capabilities are suppressed while tumor spreading is simultaneously encouraged by CD73 adenosine and A2AR activation. The anticancer effects of NK cell-based immunotherapy may therefore be enhanced by blocking the CD73-A2AR axis. A3AR agonists, on the other hand, can boost NK cytotoxicity and support the antitumor function, which is different from the effect of A2AR activation. Adenosine also makes NK cells less sensitive to ex vivo activation with IL-12 and IL-15, resulting in increased IFN- expression from CD56+ subsets. These findings challenge the idea that adenosine causes a general decrease in NK cell activity and instead point to an adenosine effect on NK cells that is dependent on the cellular environment [Zhang B. et al., 2019].

x. Macrophages

Circulating CD14+ monocytes in healthy people exhibit low levels of CD73 expression, whereas tissue-resident macrophages exhibit both CD39 and CD73. The release of ATP caused by macrophage activation by TLR activation is quickly digested by cell surface CD39. Adenosine synthesis is controlled by CD39 and CD73 expression levels. The pro-inflammatory M1 population and the anti-inflammatory M2 population are the two main subsets of macrophages. Compared to the M2 subpopulation, M1 macrophages express lower amounts of CD39 and CD73. Changes to CD39 and CD73's ectoenzyme activity may optimize how well they perform in an inflammatory environment. The CD73 blockage by APCP increased the dominance of the M1 subset in a mouse model of myocardial infection. Similar to this, M2 macrophages are reduced and the M1 fraction is increased in tumor-bearing animals when CD73 activity is blocked. However, the addition of exogenous AMP or a CD73 inhibitor has no effect on the polarization of human macrophages that have been driven by LPS and TNF- to express CD73. Additional research is needed to assess the significance of CD73 for differentiation and function of tumor-associated macrophages isolated, particularly from human tumors, in order to better understand the effects of CD73 on macrophages during tumor propagation. 2019 [Zhang B. et al]

xi. Myeloid-derived suppressor cells

Myeloid-derived suppressor cells, or MDSCs, are a heterogeneous population made up of many immature myeloid cell subsets that build up during the growth of tumors. Immune checkpoint blockade and other immunotherapies are less effective when MDSCs are present because they penetrate human malignancies. MDSCs coexpress CD73 in tumor-bearing mice, and subsequent A2BR activation encourages their growth. In a mouse melanoma model, A2BR inhibition also lowers the number of MDSCs that infiltrate tumors. A2BR agonist therapy, on the other hand, promotes MDSC tumor invasion. Human studies demonstrating greater CD39 and CD73 levels on MDSCs isolated from cancer patients compared with healthy donors lend support to these findings. In non-small-cell lung cancer patients, the coexpression of CD39 and CD73 also reveals a separate inflammatory subpopulation with enriched suppressive molecular markers. Particularly, tumor-derived TGF- activates the mTOR-HIF-1 pathway to cause CD39/CD73 coexpression on MDSCs. Additionally, MDSC CD39/CD73 aids in their immunosuppressive function and forecasts chemotherapy response. In ovarian cancer (OC) patients, metformin treatment consistently reduces CD73 expression and ectoenzyme activity on monocytic and polymononuclear MDSC subsets, which is linked to enhanced T cell-mediated antitumor immunity. Through the activation of AMP-activated protein kinase and subsequent reduction of HIF-1, metformin treatment for OC patients results in a downregulation of CD39/CD73 expression on MDSCs. In diabetic patients with OC, metformin therapy results in an increase in the antitumor activities of circulating CD8+ T cells at the same time and is associated with a longer overall survival. These findings taken together imply that inhibiting CD39/CD73-dependent MDSCs may open up fresh possibilities for future cancer immunotherapeutic approaches. 2019 [Zhang B. et al]

xii. Other immune cells & stromal cells

Long recognized as a B cell differentiation marker is CD73. Numerous studies have shown that a portion of memory B cells express CD73. IgG class switching is impaired when B cell CD73 activity from healthy controls is blocked in vitro. Despite the dearth of information regarding B cell CD73 expression and its role in cancer, a recent study found that B cells play a crucial role in delivering anticancer activity when utilizing APCP in a rat melanoma model. In the IL-33-rich tumor microenvironment, CD73 is expressed on type 2 innate lymphoid cells (ILC2s) and can be markedly enhanced on tumoral ST2+ ILC2s. It's significant to note that ILC2-derived CD73 is probably responsible for inhibiting NK cell-mediated anticancer activity [Zhang B. et al, 2019].
In most tumor microenvironments, cancer associated fibroblasts (CAFs) represent a significant stromal component. Patients with OC and nonmuscle invasive bladder cancer have a poor prognosis when CAF CD73 expression is high. OC and triple negative breast cancer in humans both have CD73-expressing CAFs. In these two investigations, CAFs improved T-reg content and survival, which was somewhat influenced by CD73 activity. Similar to the immunosuppressive effect of tumor cell CD73, CAF CD73 expression suppresses T cell-mediated antitumor immunity in a mouse model of OC. Additionally, it has been demonstrated that CD73 expression in the stroma is related with a positive disease prognosis, whereas CD73 expression in the prostate epithelium is associated with immune suppression and the advancement of prostate cancer [Zhang B. et al, 2019].

3. Regulation of CD73 Expression in Cancer

As previously discussed, CD73 has been discovered to be overexpressed in a variety of malignancies and plays crucial roles in the development of malignant tumors. How is the level of CD73 expression controlled in tumors? There are a number of mechanisms that can answer this. First off, it has been discovered that the estrogen receptor (ER) controls CD73 expression adversely in breast cancer, with ER loss considerably increasing CD73 expression. Therefore, CD73 may provide a prospective target for clinical treatment of ER negative breast cancer patients because it is more abundantly expressed in ER negative than ER positive breast cancer patients. In contrast to ER, thyroid hormones have been found to be a chemical that can boost CD73 expression in a variety of cell types that are part of the neurological and cardiovascular systems, including glioma cells, vascular smooth muscle cells, and ventricular myocytes [Zhang H-Z. et al, 2014].

Second, a hypoxic environment can stimulate CD73 expression. This is because the CD73 gene promoter contains an HIF-1 (hypoxia-inducible factor-1) binding site, known as the hypoxia response element (HRE), which increases CD73 expression when the environment is hypoxic. Accordingly, it has been discovered that HIF-1 suppression caused by antisense oligonucleotides or point mutations in the CD73 gene's HRE inhibits hypoxia-induced CD73 expression [Zhang H-Z. et al., 2014].

The third is that inflammatory factors can also control CD73 expression. Brisevac et al. calculated the impact of numerous inflammatory factors (LPS, TNF-a, IFN-gamma, and Glu: glutamate) on CD73 expression. It's interesting to note that the data showed that IFN-, LPS, and Glu could all dramatically lower CD73 expression levels, however TNF had no discernible effect on CD73 levels. Additionally, it has been noted that IFN- and IFN- have an effect that increases CD73 expression [Zhang H-Z. et al, 2014].

Epigenetic alteration controls CD73 expression as well. According to Lo Nigro et al., the methylation of CD73's CpG island in breast cancer cell lines and actual tumor tissues inhibits the protein's ability to produce itself. Similar to this, Wang et al. demonstrated that methylation-dependent transcriptional silencing decreased CD73 expression in melanoma cell lines. Additionally, methylation of the CD73 CpG islands was linked to downregulated CD73 expression in clinical melanoma cases and was related to metastatic site selectivity [Zhang H-Z. et al., 2014].

Finally, according to some additional research, CD73 expression is also regulated by the Wnt pathway, polyunsaturated fatty acids (PUFA), and intracellular cAMP. Together, our findings show that many variables and distinct processes control the expression levels of CD73. [H-Z. Zhang et al., 2014]

2. Clinical Significance of CD73 in Cancer Patients

In numerous preclinical investigations, inhibiting CD73 (and/or A2AR) restores antitumor immunity, with combined methods demonstrating greater efficacy. As a result, numerous clinical trials combining ICI therapy, A2AR antagonism, targeted therapy, and/or chemotherapy with suppressing CD73 (e.g., antibodies against CD73 or small molecule inhibitors) are under progress. In preclinical investigations, greater CD8+ T cell recruitment was linked to a sustained therapeutic effect. In the future, it will be interesting to observe if the effectiveness of CD73 and/or A2AR therapy may be further improved when utilized in early lines of therapy. Some patients who are responding can have an Adeno-signature. It may be advantageous to determine whether this signature can also be seen in pretreatment biopsies of other malignancies and perhaps primary tumors [Harvey J.B., 2020].

The ability to recognize patients who will benefit most from CD73/adenosine receptor therapy will probably depend on biomarkers or gene signatures. A dual A2AR/A2BR antagonist is now undergoing clinical trials, with an emphasis on GI tumors such esophageal cancer and CRC. Future research in GI tumors will be very helpful in figuring out if adenosine-mediated resistance to immunotherapy therapy exists at diagnosis or develops with treatment. According to preclinical research, CD73/adenosine treatment (such as A2AR deletion) frees CD8+ T cells for antitumor efficacy even against sarcomas with limited immunogenicity. The anatomical location of the tumor has no bearing on the efficacy of the therapy in these investigations. Thus, it is anticipated that the therapeutic advantage will extend to both immunogenic and non-immunogenic cancers. The development of immunotherapy techniques will depend heavily on our understanding of the mechanisms that inhibit immune cells from identifying and killing cancer cells. Poor tumor immunogenicity can be caused by a variety of factors, such as downregulation or loss of the HLA class I molecule; genetic, epigenetic, and chromosomal alterations governing the presentation and processing of surface epitopes; expression and secretion of immunosuppressive factors (e.g., PD-1, TGF-, adenosine); and the inability of cancer cells to produce novel surface epitopes that are distinct from those immune receptors It is uncertain whether CD73 expression correlates with dMMR/MSI-H in GI malignancies and whether blocking it might improve the effectiveness of immunotherapy in these tumors. Neoantigen load and tumor mutational burden have not been linked in NSCLC studies to high or low expression of CD73 [Harvey J.B., 2020].

Table 2: The clinical significance of CD73 in cancer patients adapted from [Zhang H-Z. et al, 2014]

Table 2: The clinical significance of CD73 in cancer patients.

Cancer type	Number of patients clinically assessed	Clinical implication of CD73
Colorectal cancer	358	Overexpression of CD73 is an independent poor prognostic biomarker for human CRC
Epithelial ovarian carcinoma	167	Overexpression of CD73 in epithelial ovarian carcinoma is associated with better prognosis, lower stage, and better differentiation
Gastric cancer	68	CD73 overexpression was positively correlated with differentiation of tumor, histopathology, depth of invasion, nodal status, metastasis, cancer stage, and low overall survival rate of patients

Gallbladder cancer	108	Survival time of patients with NT5E expression was significantly shorter than those without NT5E expression
Leukemia	86	Expression of CD73 was associated with leukemia subtype, differentiation, and development
Chronic lymphoblastic leukemia	229	High expression of CD73 was associated with a more aggressive and proliferation disease
Acute lymphoblastic leukemia (children)	338	CD73 expression had no prognostic value in children with acute lymphoblastic leukemia
Prostate cancer	116	Overexpression of CD73 in prostate cancer is associated with lymph node metastasis
Malignant melanoma	52	CD73 expression is epigenetically regulated in malignant melanoma and associated with metastatic site specificity
Breast cancer (stages I–III)	136	Elevated CD73 expression in stages I–III breast cancer can predict a good prognosis
Triple negative breast cancer	661	CD73 gene expression was significantly associated with a worse prognosis in TNBC patients but not in patients with luminal or HER2+ breast cancer
Luminal breast cancer	2083	
HER2+ breast cancer	487	

Numerous cancer forms have been shown to overexpress CD73, and correlative research has revealed its clinical importance (Table 2). According to research by Loi et al., patients with triple negative breast cancer (TNBC) but not those with luminal or HER2+ breast cancer had a significantly worse prognosis when CD73 expression was present. An elevated CD73 expression, however, was found to be strongly correlated with both longer disease-free survival and overall survival of breast cancer patients, according to a different retrospective study. This finding suggested that elevated CD73 expression may be able to predict a favorable prognosis in patients with stages I through III breast cancer. It should be noted that the predictive significance of CD73 expression in breast cancer is still debatable and depends on other clinical indicators. Our team is currently conducting a retrospective analysis on a larger number of clinical specimens, which will evaluate a wider range of variables, including patient age, subtype, and population, treatment, and more. [H-Z. Zhang et al., 2014]

Recently, Lu et al. evaluated the clinical importance and prognostic value of CD73 in human patients with gastric cancer. Immunohistochemistry (IHC) analysis of CD73 expression revealed that overexpression of CD73 was positively correlated with tumor differentiation, depth of invasion, nodal status, metastasis, and the cancer stage, and that the overall survival rate was low in the patients with high expression of CD73. Other investigations revealed that high levels of CD73 in patients with colorectal cancer (CRC) were associated with a poor prognosis in addition to gastric cancer. Additionally, a recent study by Xiong et al. found that CD73 expression was related to tumor development and survival in patients with gallbladder cancer [Zhang H-Z. et al., 2014]

Zhao et al. looked at the expression of CD73 in distinct leukemia subtypes in hematologic neoplasms and found that it was connected to leukemia subtype, differentiation, and development. While another study revealed that CD73 expression had no prognostic value in children (1-18 years old) with acute lymphoblastic leukemia (ALL), Serra et al. evaluated the clinical implications of CD73 in chronic lymphoblastic leukemia (CLL) and discovered that high expression of CD73 was associated with a more aggressive clinical behavior.

Yang et al. observed that upregulation of CD73 in prostate cancer was connected to lymph node metastases in other malignancies. Furthermore, Oh et al. demonstrated that overexpression of CD73 was linked to a better prognosis, a lower stage, and better differentiation in epithelial ovarian cancer. According to Wang et al., the

specificity of the metastatic site was correlated with CD73 expression in malignant melanoma. Together, these findings demonstrated the significance of CD73 as a clinical or prognostic biomarker in numerous cancer types and suggested the potential utility of CD73 for clinical diagnosis and prognosis. [H-Z. Zhang et al., 2014]

1. Autoimmune Hepatitis

The severe hepatopathy known as autoimmune hepatitis (AIH) is caused by the abnormal activation of Th17 cells as well as CD8+ and CD4+ effectors. Immune imbalance in AIH is a result of decreased T-reg counts and functional dysfunction. AIH patient-derived T-regs exhibit diminished ENTPD1/CD39 expression and are unable to inhibit eATP-mediated Th17 accumulation. Th17CD39+ cell deficiency and dysfunction have also been found in juvenile autoimmune liver disease. In this instance as well, the deficiencies in ENTPD1/CD39 and A2A expression may support and encourage cellular effector qualities. Adenosine deaminase levels are also much higher in AIH patients, and they positively correlate with scores for inflammation and fibrosis. [M. S. Longhi et al., 2019]

NKT cells are another cell type that contributes to the pathophysiology of AIH. Genetic ablation of Entpd1/Cd39 enhances eATP/P2X7-mediated NKT apoptosis and paradoxically protects against liver injury in mouse models of Concanavalin-A-induced hepatitis. As previously demonstrated in the setting of hyperoxic lung injury, extracellular purines differentially affect various cell types (T-reg vs. NKT cells) in particular pathological situations. These unexpected results show how intricate purinergic immunomodulation is, both in the liver and elsewhere. [M. S. Longhi et al., 2019]

2. Liver Fibrosis

A pathological condition known as hepatic fibrosis arises in response to persistent liver damage and chronic inflammation. Myofibroblasts, a diverse population of activated non-parenchymal liver cells and hepatic stellate cells, are what fuel the pathological process (HSC). Collagen and extracellular matrix are most likely produced primarily by these two cell types in the liver. The expression of many ectonucleotidases, including CD73 and members of the ENTPD family, is increased in HSC, portal fibroblasts, and fibrous septa. Myofibroblastic differentiation is assumed to be the cause of such levels of overexpression, which are mediated by SP1 and SMAD promoter elements [Longhi M.S. et al., 2019].

In experimental studies, CD73-deficient animals do not develop liver fibrosis, indicating that AMPase activity and adenosine synthesis play a deleterious role in the process of fibrogenesis.

There is proof that ENTPD1/CD39 regulates gut primed CD8 T-cell accumulation in the liver, avoiding biliary damage and eventual fibrosis. In this situation, elevation of the T-cell gut-tropism receptor, integrin 47, leads to an increase in hepatic CD8 T-cell numbers as a result of ENTPD1/CD39 deletion. Accordingly, stable ATP agonist or antibiotic administration, gut decontamination, and CD8 cell reduction all reduce hepatobiliary damage and fibrosis in Mdr2Cd39 mice [Longhi M.S. et al, 2019].

3. Hepatic Steatosis/Alcoholic Hepatitis

Adenosinergic actions and purinergic signals are significant regulators of metabolic illness. Increased insulin resistance and abnormal hepatic glucose metabolism are related to ENTPD1/CD39 deletion. Additionally, the expression of the A1 adenosine receptor on adipocytes affects the metabolism of fatty acids, which affects insulin resistance, diabetes, dyslipidemia, and lipolysis [Longhi M.S. et al., 2019].

The effects of ethanol-induced hepatic steatosis may therefore be mediated via adenosine receptors, particularly A1 and A2B. Adenosine can also be produced during ethanol metabolism. The severity of obesity-related non-

alcoholic fatty liver disease is closely correlated with A2AR disruption in hepatocytes and macrophages, which promotes inflammation and lipogenic processes. [M. S. Longhi et al., 2019]

4. Liver Transplant Rejection

Immune-mediated organ rejection is a potentially catastrophic side effect of liver transplantation. In allotransplantation models, liver survival is improved by upregulating and enhancing the activity of ENTPD1/CD39, which is accomplished also through exogenous injection. Additionally, the expression of ENTPD1/CD39 in liver allografts controls anti-donor effector T-cell responses and T-reg infiltration, reducing organ rejection and avoiding graft-versus-host reactions. [M. S. Longhi et al., 2019]

Extracellular nucleotides play a role in the vascular inflammation and thrombosis that lead to liver xenograft rejection.

5. Hepatocellular Carcinoma and Metastatic Liver Tumors

Hepatocellular carcinoma (HCC) is the primary liver cancer that affects adults most frequently and is the leading cause of death in cirrhotic patients. Accumulation of cellular and inflammatory metabolites, particularly eATP, which encourages the development of preneoplastic foci via P2 receptors, supports the growth and progression of HCC. Further research suggests that similar eATP-P2 receptor-mediated changes, such as the suppression of liver cell autophagy, altered metabolism, and increased proliferation, result in the development of autochthonous liver cancer in ENTPD1/CD39 mice. [M. S. Longhi et al., 2019]

Recent research has demonstrated that changes in purinergic signaling, along with immunological evasion, encourage the formation of HCC. Endothelial and T-reg cell expression of ENTPD1/CD39 promotes the growth of metastatic and transplanted hepatic tumors in mice. [M. S. Longhi et al., 2019]

Effector cell proliferation and function are inhibited by the production of adenosine by ENTPD1/CD39 produced by T-regs and myeloid derived suppressor cells (MDSC). Interestingly, HCC cells upregulate ENTPD2, which preferentially converts extracellular ATP to ADP and minimal AMP, in the presence of a hypoxic milieu, boosting the buildup and immune suppressive action of MDSC [Longhi M.S. et al, 2019].

3. The roles of CD73 in cancer

1. CD73 in human cancers

CD73 inhibits anti-tumor immune surveillance at the level of T and natural killer (NK) cells, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages by converting extracellular adenosine monophosphate (AMP) into immunosuppressive adenosine (TAMs) [JJ Kobie, 2006]

Adenosine synthesis rises in cancer due to upregulated CD73 expression in tumor cells and tumor stromal cells, which:

- ☐ Suppresses the generation of cytokines and the proliferation of T and NK cells, as well as the activity of antigen-presenting cells (APCs).
- ☐ Encourages the proliferation and suppressive action of regulatory T cells (T-reg).
- ☐ Promotes macrophage M2 polarization and MDSC stimulation.

These modifications promote illness development and tumor growth [Kobie JJ, 2006].

Purinergic signaling, which is controlled by a number of nucleotidases, has become a significant factor in the development of cancer. Numerous cancer types have been found to have elevated levels of CD73, one of the enzymes in the cascade that catalyzes the breakdown of AMP into adenosine. Expression of CD73 is controlled by a number of variables and mechanisms. A growing body of research has revealed that CD73 is an essential regulator of tumor angiogenesis, tumor immune evasion, and cancer cell migration and invasion in vitro. With its crucial involvement in cancer, CD73 has emerged as a promising therapeutic target. Recent research in mouse models showed that CD73 targeting could one day be a successful therapeutic strategy for cancer patients. The multiple functions of CD73 in the development of cancer will be outlined in this review, along with their clinical importance, their effects on tumor growth, metastasis, and angiogenesis as well as their inhibitory effects on immune response, the regulatory mechanisms that control CD73 expression, and the status of anti-CD73 cancer therapy at the time of writing [Zhang H-Z. et al., 2014].

The complex malignancy processes of tumorigenesis, progression, and metastasis in vivo involve a number of actions, such as the uncontrollable and rapid proliferation of mutant cells, the inhibition of programmed cell death, an abundance of angiogenesis, escape from immune surveillance, invasion, and colonization of distant organs. There are numerous signaling pathways that have been linked to the development of cancer. The extracellular ATP (adenosine triphosphate), ADP (adenosine diphosphate), and adenosine operate as the main signaling molecules in the purinergic signaling pathway, which has recently emerged as a key actor in the progression of cancer. Purinergic signaling is a multi-step coordinated cascade that involves nucleotide inactivation to adenosine, accelerated release of ATP/ADP, and P2 receptor-triggered signaling events. Adenosine also interacts with its own active P1 receptors to affect biological processes such cell motility, proliferation, and survival. The fact that adenosine is one of the most significant immunosuppressive regulating chemicals in the tumor microenvironment is also now clear [Zhang H-Z. et al., 2014].

A variety of cell types release nucleotides in response to various stress signals, including damage, hypoxia, inflammatory conditions, etc. Additionally, the nucleotides are hydrolyzed by the enzyme cascade as follows: ATP/ADP is converted to AMP by NTPDases, AMP is next converted to adenosine by ecto-5'-nucleotidase, and adenosine is finally converted to inosine by adenosine deaminase. Therefore, a number of ectonucleotidases that are present on the cell surface control purinergic signaling. The management of tumor growth depends on maintaining the proper balance of ATP/ADP, AMP, and adenosine. [H-Z. Zhang et al., 2014]

Breast cancer, colorectal cancer, ovarian cancer, gastric cancer, and gallbladder cancer are just a few of the cancers for which it has been discovered that CD73 is overexpressed in cancer cell lines and patient biopsies. It has also been linked to clinical traits or patient prognosis. A growing body of research has confirmed that CD73 plays a crucial regulatory role in the growth of cancer. In particular, anti-CD73 therapy has emerged as a viable strategy for the treatment of cancer patients in the future due to the good effect on tumor-bearing mouse models, although not having been studied in clinical patients. Specifically, we will discuss the clinical importance of CD73 in cancer patients, its promotion of tumor growth, metastasis, and angiogenesis, as well as its suppression of immune system function in the presence of tumor microenvironment, regulation mechanisms of CD73 expression, and potential applications of anti-CD73 cancer therapy in the future. [Zhang H-Z. et al., 2014]

Table 3 CD73 expression in different cancer cell lines [Zhang H-Z. et al, 2014]

Table 1: CD73 expression in different cancer cell lines.		
Cancer type	Cell line	Expression

Melanoma	C8161, PMW, SBCL2, SKMel28, SKMel30, KMel147, SKMel173, Mel224, WM266-4, M902-6	Higher mRNA expression than WM35 et al. because the absent of methylation
Melanoma	WM35, Mel501, Mel505, SKMel2, SKMel23, C81-61	Lower mRNA expression caused by the NT5E CpG island methylation
Melanoma	A375	High expression levels and activity
Mouse breast cancer	4T1.2, E0771	Higher expression levels than nonmetastatic variant of 4T1.2
Ovarian cancer	SKOV3	SKOV3 human ovarian cancer cells highly expressed CD73
Breast cancer	MB-MDA-231, T-47D	T-47D with lower expression of CD73 and MB-MDA-231 with higher expression of CD73
Primary Medulloblastoma	Daoy, ONS76	Daoy and ONS76 express higher levels of CD73 while D283 revealed poor expression of CD73
Metastatic Medulloblastoma	D283	
Bladder cancer	RT4, T24	Both cell lines expressed CD73

One of the characteristics of cancer has lately come to light: avoiding immune destruction. Multiple immune evasion mechanisms caused by tumors have been discovered, offering a variety of potential targets for cancer treatments. The phosphohydrolysis of extracellular ATP to adenosine can now be seen as one of the most significant immunosuppressive regulatory pathways in the tumor microenvironment given the immunological effects of CD39/CD73 and the A2A adenosine receptor (A2AR) on cancer growth and metastasis. On the growth and spread of tumors, CD73-mediated adenosinergic actions play a crucial role [Zhang B., 2012]. Extracellular AMP is dephosphorylated to adenosine by the cell surface enzyme CD73. It is widely expressed in a variety of cancers, has been linked to a pro-metastatic phenotype in melanoma and breast cancer, offers prognostic information to colon cancer patients, and has been proposed as a diagnostic tool for papillary thyroid cancer. the cancers. The primary effect of the controlled enzymatic phosphohydrolytic activity on extracellular nucleotides on CD73's biological function. Adenosine is produced from ATP by an ecto-enzymatic cascade working with CD39 (ecto-ATPase), which then sends signals via adenosine receptors. Previous research claimed that CD73 interacts with cells and the cell matrix and linked CD73 to tumor development and medication resistance. Through its ecto-enzymatic activity, CD73 expressed by tumor cells greatly reduces adaptive anticancer immunological responses, which suggests that tumor CD73-generated adenosine inhibits T-cell immunosurveillance of mouse malignancies. Overall, the A2AR and extracellular adenosine form a crucial axis in tumor immune evasion. 2012 [Zhang B.]

Finally, tumor development and metastasis were markedly inhibited in vivo by pharmacological inhibition of CD73 using the selective inhibitor APCP or an anti-CD73 monoclonal antibody. Inhibiting both tumor and host CD73 is necessary for CD73 blockage to have the best anticancer effect. Tumor remission was achieved by combining APCP or anti-CD73 monoclonal antibody therapy with adoptive T cell therapy. As a result, all three separate research teams reached the same conclusion: targeting CD73 can be used therapeutically to prevent tumor development and metastasis. Lack of CD73 activity in host cells represents a novel mechanism governing anti-tumor immunity and tumor progression. The main factor in cancer patient mortality is metastasis, a deadly trait of malignancy malignancies. Therefore, finding molecules that encourage metastasis is crucial for developing target medications and conducting anticancer research. CD73 has been linked to tumor metastasis in both clinical patients and experimental animals, according to growing findings. [H.-Z. Zhang et al., 2014]. In MB-MDA-231 cells, researchers discovered a positive correlation between CD73 expression and EGFR expression, and that EGFR expression could be lowered by silencing CD73 expression. By controlling EGFR expression, CD73 encouraged tumor cell migration and invasion [Zhang H-Z. et al., 2014].

According to Xiong et al., CD73 is an important regulator of the epithelial-mesenchymal transition (EMT) in gallbladder cancer, suggesting that CD73 may encourage tumor cell motility and invasion by generating EMT. It's interesting to note that CD73's twin activities appear to play independent roles in cell contact and movement on the ECM. [H-Z. Zhang et al., 2014]

Several retrospective investigations on clinical cancer patients found that metastasis of gastric cancer, prostate cancer, and malignant melanoma were all correlated with tumor CD73 overexpression. Together, the data showed that both host and tumor CD73 play a substantial role in tumor metastasis. Importantly, host CD73 deficiency has a protective impact against tumor metastasis that is linked to an increase in endogenous antitumor immunity. [H.-Z. Zhang et al., 2014]. Particularly in cancer-related conditions, CD73 helps endothelial cells produce new blood vessels. Cancer cells need a lot of nutrition and oxygen to proliferate unchecked. A high angiogenesis density may provide the necessary conditions for the development of tumor cells. Blood can carry tumor cells into distant organs by entering into immature arteries. Therefore, a lot of tumor angiogenesis is required for the growth and metastasis of cancer. [H-Z. Zhang et al., 2014]

The significance of CD73 expression during tumor angiogenesis and the impact of anti-CD73 mAb treatment on angiogenesis were both examined by Allard et al. While host-derived CD73 is necessary for the best angiogenic responses to VEGF, tumor-derived CD73 increases VEGF synthesis by tumor cells. The angiogenesis of tumors developed in CD73-deficient mice appeared to be reduced. These findings confirm that tumor and host CD73 cooperate to promote angiogenesis in vivo when there is a tumor. [H-Z. Zhang et al., 2014]

Bevacizumab is one of numerous inhibitors of angiogenesis that have been approved by the FDA and utilized in clinical trials as a result of the tumor's ability to promote angiogenesis' role in growth and metastasis (i.e., anti-VEGF mAb). Patients who initially respond well to antiangiogenic therapy, however, eventually develop resistance. The aberrant activation of the VEGF pathway may be a factor in the processes. As was already established, CD73 can enhance VEGF expression, which suggests that CD73 contributes to acquired resistance to anti-VEGF therapy. [H-Z. Zhang et al., 2014]

2. CD73 in tumor immunity

In vitro, it has been discovered that overexpressing CD73 encourages the growth of cancer cells. According to Zhi et al., expression inhibition of CD73 by shRNA can stop breast cancer cells (MB-MDA-231) from proliferating by causing cell-cycle arrest and cell death. Treatment with APCP (, -methylene adenosine-5’-disphosphate), a particular inhibitor of CD73 enzymatic activity, can also, in a dose-dependent manner, reduce the growth of cancer cells. CD73 stimulates the development of cancer cells because it produces adenosine as a result of its enzymatic activity. [H-Z. Zhang et al., 2014]

The pro-proliferative action of CD73 on cancer cells in vitro may be mediated by other molecules other than adenosine, such as EGFR, a crucial molecule implicated in cell growth. Recently, Zhi et al. discovered that CD73 regulates the expression and phosphorylation of EGFR in human breast cancer. [H.-Z. Zhang et al., 2014].
Overexpression of CD73 in tumor cells can facilitate the development of subcutaneous tumors in mice models in vivo. According to research by Zhi et al., subcutaneous tumors created by injecting pcDNA-NT5E transfected MCF-7 cells into nude mice developed more quickly than those created by original MCF-7 cells. Furthermore, CD73 downregulation by siRNA has consistently been shown to reduce tumorigenicity in mouse xenograft models. Notably, host CD73, in addition to the CD73 found in tumor cells, is crucial for the in vivo development of cancer. Yegutkin et al. looked at how host CD73 affected the development of tumors in melanoma models and discovered that mice lacking CD73 had primary tumors that were considerably less aggressive. When considered collectively, it may be necessary to target both host and tumor CD73 for the best anticancer impact of CD73

blocking medication [Zhang H-Z. et al., 2014]. This is because both host and tumor CD73 contribute to initial tumor growth in vivo.

Most human solid tumors express CD73 more strongly than other tumor types. Its expression and activity are closely linked to the metastasis and invasion of the tumor. CD73 on tumor cells can produce enough extracellular adenosine to mediate immune evasion, promoting the growth and spread of the tumor. Multiple CD73-deficient tumor models have been used to further demonstrate the significance of CD73 on tumor cells as opposed to host cells in carcinogenesis. In addition to being immune-regulated by tumor cells, CD73 has a variety of effects on carcinogenesis, including metastasis, adhesion/migration, proliferation, and angiogenesis. By controlling the cell cycle, apoptosis, and signaling pathways such EGFR, -catenin/cyclin D1, VEGF, and AKT/ERK, it encourages the development of tumor cells [Zhang B. et al., 2020].

In addition to its enzymatic activity, CD73 can encourage stemness as well as cell-to-cell adhesion, migration, and cancer cell invasion. It's interesting to note that tumor angiogenesis requires CD73 on both host and tumor cells. Further supporting the possible use of adenosine blocking drugs to decrease pathological lymphangiogenesis in malignancies and stop tumor dissemination is the fact that CD73-A2AR signaling is crucial for tumor-associated lymphangiogenesis. Additionally, according to two recent studies, intrinsic CD73 found in cancer cells sped up metastasis by promoting EMT via the PI3K/AKT signaling route and the RICS/Rho GTPase signaling circuit, respectively. To back this viewpoint, CD73 expression is frequently linked to a worse prognosis and a subpar response to treatment [Zhang B. et al, 2020].

However, CD73 expression has been found to be associated with a good prognosis and is not always elevated in malignancies. In fact, human hepatocellular carcinoma (HCC) has been found to have aberrantly glycosylated CD73 and a human-specific CD73 isoform (CD73s), which results in the functional suppression of tumor CD73. In advanced stage prostate, laryngeal, and high grade colon carcinomas, CD73 was likewise downregulated. Comparing poorly differentiated and advanced stage endometrial carcinomas to normal and well-differentiated early stage tumors, lower levels of CD73 expression were found, while higher CD73 expression was linked to a better overall survival. Because of the non-tumor promoting actions mediated by CD73, the role of CD73 in malignancies appears to be multifaceted [Zhang B. et al, 2020].

The existence of a soluble version of CD73 (sCD73) and its elevated levels in the plasma of cancer patients as compared to healthy people are also supported by evidence. High levels of sCD73 enzyme activity in serum, prior to nivolumab (anti-PD-1 Ab) treatment, were found to be associated with poor survival of metastatic melanoma patients, despite the fact that the role of sCD73 is less well understood [Zhang B. et al., 2020]. This suggests that sCD73 may be a prognostic marker for cancer immunotherapy.

Intriguingly, CD73 and CD39 were discovered on exosomes isolated from patients with mesothelioma, and CD73+ exosomes inhibited immune cell activity. Additionally, CD73 expression on dendritic cells (DC) was induced by prostate cancer cell-derived exosome, preventing T cell activity. As a result, tumor cells or their exosome-derived products use CD73 to inhibit the immune system in an adenosine-dependent manner [Zhang B. et al, 2020].

Extracellular adenosine concentrations in the mouse tumor microenvironment have recently been found to be elevated. Extracellular adenosine can enter the tumor microenvironment passively or voluntarily from a number of sources. Extracellular adenosine is most likely created by the enzymatic breakdown of extracellular ATP or by passive diffusion or active transport of intracellular adenosine. An increase in intracellular AMP, an accumulation of intracellular adenosine, and a subsequent transport or diffusion of intracellular adenosine into the extracellular space are linked to local tissue hypoxia that occurs after injury to endothelial cells and microcirculation and the cessation of regular blood and oxygen supply. The degree of ischemia/necrosis in tumor tissues determines how much adenosine is produced; nevertheless, this method may not have a significant impact on the production of extracellular adenosine [Zhang B., 2010].

The primary result of the controlled enzymatic phosphohydrolytic activity on extracellular nucleotides is the biological effects of CD73 (ecto-5'-NT). Adenosine is produced from ATP via this ecto-enzymatic cascade and CD39 (ecto-ATPase), which then activates adenosine receptors. Despite being highly dependent on the availability of extracellular AMP, production of extracellular adenosine by CD73 is likely the predominant method of adenosine generation in epithelial cells, in contrast to the intracellular generation of adenosine from cytosolic pools of adenine nucleotides catalyzed by cytosolic 5'-NT in the heart. Importantly, elevated enzymatic activity that can facilitate the generation of extracellular adenosine is associated by a considerable up-regulation of CD73 in malignant tissues [Zhang B., 2010]. Through surface CD73 enzymatic activity, tumor cells themselves contribute to the increased levels of adenosine in the tumor microenvironment. The local tumor microenvironment is likely to produce high levels of tumor CD73 expression. However, there are inconsistent observations in the literature about how proinflammatory cytokines affect CD73 expression. Recent research has identified a regulation route for CD73 on epithelial cells that is dependent on hypoxia inducible factor-1 (HIF-1). The current data thus supports the idea that hypoxia, HIF-1, and CD73 convert AMP to adenosine in a sequential pattern that results in increased extracellular adenosine levels in the tumor [Zhang B., 2010].

Adenosine effectively suppresses a number of T cell responses, such as antigen-induced proliferation, secretion of IL-2 and proinflammatory cytokines like interferon and TNF-, up-regulation of CD25, induction of cytolytic effector molecules like perforin and Fas ligand, adhesion of killer lymphocytes to tumor cells, and granule exocytosis by CTL. Adenosine and its equivalents can also inhibit the function of NK and LAK cells [Zhang B., 2010]. Thus, it is reasonable to assume that adenosine may constitute a significant portion of the so-called immunological barrier, resulting in a failure to mount an efficient antitumor immune response, given the strong immunosuppressive properties of adenosine and its presumed high concentration in solid tumors. We investigated the regulating function of tumor CD73 in antitumor T cell immunity since many tumor cells express functional CD73. In vitro and in vivo, extracellular adenosine produced by tumor CD73 suppressed the antitumor T cell response's activation phase and effector phase while promoting T cell apoptosis [Zhang B., 2010]. Additionally, siRNA-mediated CD73 knockdown on tumor cells fully restored the effectiveness of adoptive T cell therapy and resulted in long-term tumor-free survival in tumor-bearing mice, indicating that tumor CD73-mediated immune suppression through its enzymatic activity significantly contributes to cancer immune evasion. In agreement with our findings, a separate study found that blocking CD73 with an anti-CD73 monoclonal antibody (mAb) induced adaptive antitumor immunity and prevented the spread of breast tumors [Zhang B., 2010].

4. Prognostic significance

A glycosylphosphatidylinositol (GPI) anchored cell surface protein with numerous functions in tumor processes is CD73, which is encoded by the NT5E gene. Previous research suggested that CD73 might be useful for predicting the prognosis of a variety of solid tumors, although the findings were less clear-cut. To precisely assess CD73's prognostic function in solid tumors, a thorough meta-analysis was carried out. These studies demonstrated the usefulness of CD73 as a predictive biomarker in solid tumors and the relationship between CD73 overexpression and inverse OS or DFS. However, further study is still required [Wang R. et al., 2017] to determine the predictive value and target therapy for clinical practice. A lymphocyte differentiation antigen and an adhesion molecule for lymphocytes adhering to endothelium were the original definitions of CD73. Recent research suggested that several types of solid malignant tumors overexpressed CD73 (i.e., breast cancer, colorectal cancer, prostate cancer, ovarian cancer, and gallbladder cancer). Tumor hypoxic microenvironment and several soluble inflammatory agents, such as type I IFNs, TNF-, IL-1, TGF-, and Wnt activators, drove CD73 overexpression. On the other hand, estrogen receptor (ER) expression has also been shown to adversely affect CD73 expression in breast cancer. A range of physiological reactions, as well as the growth of cancer, are controlled by CD73, which catalyzes the conversion of adenosine (ADO) from AMP released to the extracellular environment [Wang R. et al, 2017].

In a tumor setting, CD73 serves both enzymatic and nonenzymatic purposes. The proliferation, angiogenesis, and death of tumor cells are all crucial nonenzymatic processes for which CD73 is essential. Therefore, CD73 plays a key role in tumor metastasis. According to the enzymatic functions, adenosine produced by CD73 is crucial for tumor immune tolerance. According to Wang R. et al. (2017), extracellular ADO can have an impact on the tumor immunological microenvironment through a variety of channels.

1) Reducing the cytotoxic activity of effective immune cells: ADO inhibits NK cells and greatly lowers CD8+ T cells homing by interfering with the process of granule exocytosis and decreasing NK cells' capacity to adhere to tumor cells.

2) Increasing immunosuppressive effects: Through A2A and A2B receptors, ADO suppresses M1 macrophage activation and enhances M2 macrophage polarization. Damage-causing tumor immune surveillance is greatly aided by myeloid-derived suppressor cells (MDSC). By activating the A2B receptors on myeloid precursor cells, ADO encourages the growth and accumulation of MDSC in the tumor microenvironment.

ADO binding to the A2B receptor can change the phenotype of dendritic cells, reduce the amount of tumor antigen presentation, and boost the production of vascular endothelial growth factor (VEGF). This results in the induction of anomalous differentiation and weakens the function of antigen-presenting cells.Together, the CD73-adenosinergic pathway plays a role in modulating the immune system and the tumor itself to produce a tumorigenic milieu [Wang R. et al., 2017].

Numerous in vitro investigations showed that the expression of CD73 was linked to tumor growth, invasiveness, angiogenesis, metastasis, and resistance to therapy. However, the following findings [Wang R. et al, 2017] indicate that the prognostic role of CD73 in various human solid tumors is debatable. Immunosuppressive CD73-adenosine signaling in the tumor microenvironment may be linked to aggressive renal cell carcinoma (RCC). By using nephrectomy samples, researchers have examined the prognostic significance of CD73 protein expression in RCC and established a correlation between the transcript levels of CD73 (ecto-5'-nucleotidase (NT5E), CD39 (ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1)), and A2 adenosine receptor (A2AR; ADORA2A) and markers of angiogenesis and The management of metastatic renal cell carcinoma (mRCC) has greatly improved thanks to immune checkpoint inhibitors that target the programmed cell death-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) pathways [Harshman C. et al, 2020].

Ecto-5'-nucleotidase (NT5E)-encoding CD73 and ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1)-encoding CD39 mediate the rate-limiting step in the successive dephosphorylation of extracellular ATP to adenosine. Hypoxia-inducible factor (HIF)-1 greatly increases CD73 expression on tumor cells and immune cells that have invaded the tumor. G protein-coupled A1, A2A, A2B, and A3 receptors bind to and are activated by the adenosine produced by CD73 and CD39. Through the growth of regulatory T cells (T-regs) and myeloid-derived suppressor cells (MDSCs), as well as the conversion of tumor-associated macrophages into the immunosuppressive M2 phenotype, enhanced adenosine signaling attenuates the anticancer immune response in preclinical models. Through enhanced vasodilation, the release of proangiogenic substances like VEGF, and the attraction of endothelial progenitor cells to the tumor microenvironment, adenosine signaling may also promote angiogenesis. In 2020, Harshman C. et al.

After controlling for other prognostic factors such tumor grade and AJCC stage in patients with localized RCC, CD73 expression was independently associated with worse prognosis and also corresponded with unfavorable histologic characteristics. Patients with both localized and advanced RCC may be affected by aggressive disease due to CD73-adenosine signaling. These findings offer convincing justification for targeting this route not only in

mRCC but also as perioperative therapy in patients with localized illness and high CD73 expression [Harshman C. et al, 2020] given that multiple medicines targeting this pathway are in early clinical development.

Granulocytic MDSC recruitment and proliferation are similarly mediated by adenosine signaling. A possible biomarker of CD73-adenosine signaling could be a gene expression signature that includes genes encoding CXCR2 ligands (such as CXCL1, CXCL2, CXCL3, CXCL5, and CXCL6) and mediators of neutrophil and MDSC biology (ILB, IL1B, and PTGS2). Several genes from the adenosine signature are included in the myeloid-inflammation gene signature that was employed in our analysis (CXCL1, CXCL2, CXCL3, CXCL8, and PTGS2). As monotherapy or in combination with licensed anti-PD-1/L1 therapies, a number of drugs that target the CD73-adenosine pathway are being tested in clinical trials for a variety of malignancies, including mRCC. Independent of stage and grade, high CD73 expression predicts significantly lower survival outcomes. One prospective to investigate is the immunosuppressive and proangiogenic CD73-adenosine pathway in RCC [Harshman C. et al, 2020].

Solid organ transplantation entails ischemia-reperfusion damage, which increases the donor organ's immunogenicity and raises the risk of rejection and graft loss. B cells are now increasingly understood to play a part in these pathogenic processes, despite the fact that T cells have historically been thought to be the ones orchestrating them. These cells are potentially interesting therapeutic targets due to the recent identification and characterisation of B cell subtypes, the recognition of dual but opposing actions on T cells, and the suspected participation in allograft rejection [Zhou P. et al., 2018]. These significant immune cell subsets exhibit the ectonucleotidase CD73, which is involved in the production of adenosine. Both B and T cell activity depends on adenosine signaling, which is also protective against both warm and cold ischemia injury and possesses immunomodulating capabilities. Adenosine receptor agonists and antagonists are now being investigated in non-transplant human trials, however focused therapy is challenging due to the widespread nature of adenosine receptors. Adenosine receptor agonists may potentially lessen the effects of ischemia-reperfusion injury, subsequent acute rejection, and graft failure when administered to the donor organ prior to implantation [Dwyer K.M. et al, 2014].

2. Strategy for prediction and prognosis

1. Role of CD73 in Disease: Promising Prognostic Indicator and Therapeutic Target

The rate-limiting enzyme for the production of adenosine is ecto-5'-nucleotidase (NT5E), whose expression in numerous cells is greatly altered by inflammation and hypoxia. When there is inflammation, CD73 guards the integrity of the endothelium barrier and prevents leukocyte trafficking. Moreover, CD73 stimulates M2 macrophages (anti-inflammatory phenotype). Additionally, T-reg cells express CD73, which uses adenosine to mediate immunological suppression [Zhou P. et al., 2018]. An important regulator of inflammation and immunity is CD73. Numerous diseases, including autoimmune disorders, ischemia-reperfusion damage, arterial calcifications, and atherosclerosis, are associated with its expression. Many malignancies have an overexpression of CD73. Its expression is strongly correlated with angiogenesis, metastasis, tumor growth, poor prognosis, and treatment resistance. As a result, CD73 may be exploited as a therapeutic target and prognostic marker in conditions like cancer [Zhou P. et al., 2018].

In several tumor forms, a clinical correlation between high CD73 expression and poor prognosis has been clearly established. It has been clearly established that T-regs and the vasculature both contribute to the overall CD73-

mediated immunosuppression. However, the role of CD73 activity in CAFs, a significant stroma population in the TME linked to poor survival for a variety of tumor types, is still poorly understood [Yu M. et al., 2020].

2. Role of CD73 in Disease: Promising Prognostic Indicator and Therapeutic Target

The rate-limiting enzyme for the production of adenosine is CD73, also known as ecto-5'-nucleotidase (eN, NT5E, EC3.13.5), which is expressed on a variety of cells. Hypoxia and inflammatory conditions have a substantial impact on its expression. When there is inflammation, CD73 guards the integrity of the endothelium barrier and prevents leukocyte trafficking. Moreover, CD73 stimulates M2 macrophages (anti-inflammatory phenotype). Additionally, T-reg cells express CD73, which uses adenosine to mediate immunological suppression [Zhou P. et al., 2018]. An important regulator of inflammation and immunity is CD73. Numerous diseases, including autoimmune disorders, ischemia-reperfusion damage, arterial calcifications, and atherosclerosis, are associated with its expression.
Many malignancies have an overexpression of CD73. Its expression is strongly correlated with angiogenesis, metastasis, tumor growth, poor prognosis, and treatment resistance. As a result, CD73 may be exploited as a therapeutic target and prognostic marker in conditions like cancer [Zhou P. et al., 2018].

Although ecto 5'-nucleotidase [cluster of differentiation (CD)73] plays crucial roles in a number of cancer types, it is still unclear how it expresses itself in squamous cell carcinoma (SCC). The purpose of the current study was to look at CD73 expression in SCC. In 113 patients with oral SCC, immunohistochemistry was used to evaluate CD73 expression (OSCC). A statistical analysis was done to determine the relationship between patient clinicopathological characteristics, overall survival (OS), and disease free survival (DFS) times and CD73 expression. 58.4% (66/113) of OSCC patients had CD73 expression, with the immunostaining primarily localized in the cytomembrane and a small amount in the cytoplasm [Zhang C-P. et al., 2016]. A statistical analysis showed that patients with bigger tumors had greater CD73 expression (P=0.021). Clinical stage and CD73 upregulation were substantially correlated (P=0.047). Additionally, immunohistochemical labeling revealed an inverse correlation between the DFS (P=0.002) and OS (P=0.002) times and CD73 overexpression. An independent predictive predictor for poor DFS and OS was CD73 expression, according to multivariate Cox regression analysis (P=0.018 and P=0.021, respectively). The clinical importance and prognostic value of CD73 in patients with OSCC are being assessed for the first time in the current investigation. According to the research, CD73 may serve as a prognostic indicator for OSCC [Zhang C-P. et al., 2016]. The mechanisms of cancer occurrence and progression involve both the enzymatic and non-enzymatic functions of CD73 [Zhang C-P. et al., 2016]. Breast cancer, prostate cancer, bladder cancer, and malignant melanoma are just a few of the cancers that have been linked to increased expression of CD73. Additionally, it has been proposed as a diagnostic factor in papillary thyroid carcinoma and has predictive relevance for individuals with colon cancer. However, very little is known about how CD73 expression on tumor cells affects a patient's chance of survival when they have SCC. In the present investigation, CD73 expression was evaluated in relation to clinicopathological traits in OSCC patients, including disease-free survival (DFS) and overall survival (OS) time [Zhang C-P. et al., 2016].

A growing body of literature has highlighted the role of CD73 in the development of cancer and overexpression of CD73 has been seen in a variety of cancer types. For instance, triple negative breast cancer patients' prognoses were considerably worsened by overexpression of CD73. In contrast, a number of retrospective investigations found a clear correlation between increased CD73 expression and better clinical outcomes in breast cancer patients. Additionally, Lu et colleagues looked at the expression status of CD73 in gastric cancer and found that it was strongly correlated with cancer stage, depth of invasion, and metastasis. Low OS was also seen in patients with overexpression of CD73, according to their findings [Zhang C-P. et al., 2016]. A poor prognosis in colorectal cancer and gallbladder cancer is statistically linked with high levels of CD73, according to a number of studies. Zhao et al. reported the CD73 status in a variety of leukemia subtypes and discovered a correlation between the

CD73 status and differentiation and leukemia subtype. Additionally, a retrospective study found that individuals with epithelial ovarian cancer who had better prognoses, lower stages, and better differentiation were more likely to have CD73 overexpression, which was positively connected with lymph node metastases in prostate cancer. Malignant melanoma showed similar outcomes. When considered collectively, the aforementioned results show that CD73 is a substantial molecular prognosticator in a variety of cancer types [Zhang C-P. et al., 2016]. The most significant prognostic indicators when assessing the survival of OSCC patients are the T stage and clinical stage. At the molecular level, the recent findings supported the T stage and clinical stage. A significant molecular prognostic marker in early T stage (T1/T2) or early clinical stage (I/II) OSCC, CD73 may also be. [C-P. Zhang et al., 2016]

3. CD73 and Drug Resistance

Overexpression of CD73 has been discovered to be related to anticancer drug resistance in addition to being a clinical and prognostic marker in cancer patients. Actually, Ujhazy et al. were the first to note that higher CD73 levels were coexpressed with the chemoresistant phenotype of cell lines (MDR positive) back in 1996. Recently, Quezada and his colleagues discovered that the vincristine resistant phenotype of glioblastoma multiforme (GBM) cells may be reversed by inhibiting CD73 activity or reducing CD73 expression by siRNA [Zhang H-Z. et al, 2014].

Recently, Loi et al. examined the relationship between CD73 expression level and the percentage of pathologic complete responses (pCRs) in triple negative breast cancer patients receiving preoperative chemotherapy that exclusively included anthracyclines. Their findings demonstrated a substantial correlation between reduced CD73 expression and a higher pCR rate. Additionally, they showed that CD73 overexpression in tumor cells provided chemoresistance to doxorubicin reliant on the activation of A2A adenosine receptor by employing mice models of breast cancer. Unsurprisingly, CD73-overexpressing tumors in mouse models with targeted inhibition of A2A adenosine receptors could recover their doxorubicin sensitivity [Zhang H-Z. et al, 2014].
Additionally, despite the rising popularity of cancer immunotherapy, the clinical results are still only somewhat beneficial due to immune escape mechanisms. CD73 is unquestionably engaged in immunotherapy resistance as a crucial regulatory molecule of immune escape in vivo. Therefore, for these patients with high CD73 levels, combining anti-CD73 treatment with chemotherapy or immunotherapy may be a successful strategy given the association between CD73 and resistance to several anticancer therapies. Future CD73 expression in cancer patients may be used as a detectable gene marker to choose and use appropriate medications for cancer treatment in the emerging era of individualized cancer therapy. [H-Z. Zhang et al., 2014]

3. Strategy for therapeutic opportunities

Although medications that target adenosine receptors show enormous potential in treating a number of disorders, specificity and pharmacodynamics have proven to be difficult. It might be possible to regulate extracellular adenosine concentrations as an alternative to agonists or antagonists that modulate adenosine receptor signaling [Colgan SP, 2006]. However, it might be able to control these metabolic processes when the main pathway includes the extracellular metabolism of ATP and/or AMP. For instance, it has been demonstrated that the drugs methotrexate and sulfasalazine enhance extracellular adenosine. It has been demonstrated that these adenosine increases both in vivo and in vitro include a CD73-mediated process. For instance, in inflammatory models, soluble 50-nucleotidase enhances vascular barrier function and reduces neutrophil accumulation [Colgan SP, 2006]. Therapies targeted at CD73 have not been extensively developed. In some situations, it may be necessary to inhibit the production of adenosine (for instance, by inhibiting CD73). As mentioned above, adenosine may encourage water transfer through intestinal epithelia and the signs of

secretory diarrhea during enteric pathogen infection. CD73 inhibitors, such as APCP, may work well as antidiarrheals for various uses. In the same manner, CD73 inhibitors might be advantageous for treating pulmonary edema brought on by viral pneumonia or inflammation. The regulated modulation of CD73 activity may have an impact on angiogenesis given the known relationship between angiogenesis and activation of the adenosine A2 receptor. For instance, systemic injection of Physiologic functions for CD73 357 and/or targeting of CD73 inhibitors may be advantageous for preventing tumor angiogenesis, which is currently a topic of great attention in the field of cancer research [Colgan SP, 2006].

Given the well-known link between hypoxia and the tumor microenvironment, where hypoxia and HIF-1 activation are strong transcriptional triggers for CD73 expression, this is very intriguing. It makes sense that many cancers could overexpress CD73 in such conditions. Consequently, CD73's inhibition of adenosine synthesis may be a therapeutic target for preventing tumor angiogenesis and metastasis. Research is being done to test this theory through experiments. [SP Colgan, 2006]

1. *Role of CD73 in Disease: Promising Prognostic Indicator and Therapeutic Target*

Facts supported by scientific evidence include the following:

- ☐ Worse clinical outcomes are frequently linked to tumor microenvironment CD73 expression levels that are high.
- ☐ Several immune checkpoint inhibitors that target CD73 or A2AR are presently being created and are being put through preliminary clinical trials.
- ☐ Early research points to the potential relevance of assessing sCD73 activity in cancer patients' serum or plasma to forecast the effectiveness of immune checkpoint therapy.

The high expression level of CD73 within the tumor microenvironment is frequently linked to worse clinical outcomes in a variety of human cancers, including melanoma, breast cancer, acute lymphocytic leukemia, CLL, glioblastoma, head and neck cancers, high-grade serous OC, endometrial cancer, colorectal cancer, prostate cancer, bladder cancer, gastric cancer, kidney cancer, and pancreatic carcinomas. Recently, 122 samples from the BIG 02-98 adjuvant Phase III clinical study have added support to the idea that increased CD73 expression in the resected tumor is associated with a worse prognosis. Additionally, CD73 expression in those samples is correlated with a diminished anti-tumor immune response. Indicating a putative adaptive resistance mechanism, CD73 overexpression is also seen in melanoma patients who are responding to adoptive T-cell transfer or immune checkpoint inhibition [Zhang B. et al, 2019].

It's interesting to note that the tumor microenvironment's CD73 and A2AR activities are not redundant. Combining CD73 with A2AR coinhibition increases antitumor activity and is more effective than using either agent alone. In order to effectively limit tumor genesis, development, and metastasis, these interdependent pathways must be targeted in combination [Zhang B. et al., 2019]. Importantly, the plasma shows evidence of soluble CD73 (sCD73) enzyme activity. Increased basal levels of the sCD73 enzyme activity in the blood before beginning nivolumab treatment are linked to lower response rates to therapy in patients with metastatic stage IV melanoma, suggesting a potential value of monitoring sCD73 activity in the serum from cancer patients to anticipate the benefit of immune checkpoint therapy. It's interesting to note that while soluble ADA (sADA) promotes the conversion of adenosine to inosine, sCD73 and alkaline phosphatase catabolize AMP to adenosine. Additionally, serum total sADA activity is noticeably increased in breast cancer patients compared to healthy persons. The expression patterns of these plasma purine-metabolizing enzymes that cause higher plasma levels of the anti-inflammatory amino acid adenosine in cancer patients are still unknown, though. More studies are

still required to determine whether sCD73 and/or sADA will be effective as serologic prognostic biomarkers in the future [Zhang B. et al, 2019].

A promising new treatment strategy for cancer immunotherapy is the adenosinergic pathway. Ecto-5-nucleotidase CD73 controls the hydrolysis of AMP to produce immunosuppressive adenosine (ADO) in this pathway, which is a unique function. ADO levels are raised as a result of CD73 overexpression, which is associated with poor patient prognosis. As a result, one possible method of treating cancer is by inhibiting CD73 in order to lower the level of ADO. We created a number of new monophosphonate small-molecule CD73 inhibitors based on the binding mechanism of adenosine 5'- (methylene) diphosphate (AOPCP) with human CD73. Among them, OP-5244 was found to be a very effective and readily absorbed CD73 inhibitor [Xiaohui Du. et al., 2020].

2. The ectonucleotidases CD73: Novel checkpoint inhibitor targets

The potential to create anti-CD73 therapy for numerous human cancers has been made possible by the growing functions of CD73 in tumor growth and metastasis, particularly as a critical immunosuppressive element in tumor microenvironment. In this context, growing evidence from studies using monoclonal antibodies or small molecule inhibitors that specifically target CD73 in mouse tumor models suggests that targeted CD73 therapy is a critical strategy for the efficient management of tumor growth and metastasis [Zhang H-Z. et al., 2014].

The presence of CD73 in the tumor environment has been linked to pro-metastatic phenotypes and/or poor clinical outcomes. By lowering adenosine (Ado) buildup and raising levels of ATP, which has immunostimulatory qualities, blocking CD39 and CD73 may boost anti-tumor immunity. Recently, it was discovered that inhibiting CD73 enzymatic activity increased the anti-tumor effectiveness of immune checkpoint inhibitors. Additionally, we demonstrate that in vivo inhibition of the ATP/Ado pathway boosted the anti-tumor activity of immune checkpoint medicines (such as PD-1 and CTLA-4) and chemotherapeutic drugs like oxaliplatin in CD39 knockout mice. CD73 is primarily expressed by tumor cells, while CD39 is commonly up-regulated on tumor infiltrating lymphocytes (TILs) compared to PBMC or nearby non-tumor tissue, according to immunohistochemistry (IHC) and flow cytometry labeling. Blocking antibodies (Abs) against human CD39 (IPH52) and CD73 (IPH53) were developed with novel features for cancer immunotherapy, potently inhibiting the enzymatic activity of both the soluble and membrane-associated versions of their respective targets. In the presence of ATP and immune cells that express CD39 and CD73, both Abs effectively counteract adenosine-mediated T cell suppression in vitro [Zhang B. et al., 2020]. The anti-CD39 IPH52 Ab boosts T cell proliferation and dendritic cell (DC) activation in vitro, most likely via preserving high ATP concentrations in the extracellular compartment. The anti-CD73 IPH53 Ab, which is currently in phase I clinical development, is more effective than benchmark Abs for blocking membrane- and soluble-associated CD73 enzymatic activity as well as reversing AMP-mediated T cell suppression. Finally, we demonstrated that the suppression of immune cells in the presence of ATP is strongly reversed when IPH52 and IPH53 Abs are combined at suboptimal dosages. Together, these findings provide evidence in favor of the need for anti-CD39 and anti-CD73 neutralizing Abs to be developed clinically for use in cancer immunotherapy, maybe in conjunction with chemotherapy or immune checkpoint inhibition. 2018 [Paturel C. et al]

The Sitkovsky group reported ground-breaking research on how A2AR shield malignancies from effector T cells with anticancer capabilities in 2006. Over the past 10 years, growing data has validated the therapeutic promise of focusing on the CD73-A2AR axis in a variety of cancer types based on the initial findings. While we reported that CD73 small molecule inhibitor therapy with APCP slowed the course of OC and increased animal subject survival, Stagg et al first's research demonstrated that CD73 mAb treatment prevented breast tumor growth and metastasis. Using various animal models, these two independent groups subsequently showed that host and tumor cell CD73 are both involved in tumor growth and metastasis, emphasizing the need for effective anticancer action to target both tissue compartments. 2019 [Zhang B. et al]

In addition to immune checkpoint-targeted therapies, agonistic anti-4-1BB therapy combined with CD73 inhibition can result in tumor regression in many animal cancer models. It's interesting that anti-4-1BB therapy preferentially activates CD73-deficient effector T cells to suppress tumor growth. However, in TGF-rich tumors that maintain the expression of CD73 on intratumoral CD8+ T cells, the efficacy of agonistic anti-4-1BB treatment is reduced. Mechanistically, the activation of STAT3 and the reciprocal regulation of TGF- and 4-1BB ligation in simultaneous CD8+ T cell activity define the effectiveness of anti-4-1BB therapy. Anti-GITR or anti-OX40 therapy selectively promotes CD73-negative CD8+ T cell immunity, much as anti-4-1BB therapy. Accordingly, CD73 inhibition and anti-GITR combo therapy has resulted in tumor regression. However, more research is needed to fully understand how CD73-mediated adenosinergic action contributes to the resistance mechanism of immunostimulatory agonistic cancer therapy [Zhang B. et al., 2019].

Anti-CD73 mAb therapy increases the efficacy of anti-ErbB2 mAb for treating engrafted or spontaneous primary tumors and lung metastases in immunocompetent mouse models of HER2/ErbB2-driven breast cancer. Increased CD73 expression was observed in melanoma patients with advanced BRAF mutation+ disease, according to a recent study. In a mouse melanoma model, the combination of anti-BRAF, MEK, and A2A receptor inhibitor treatment significantly reduces tumor start and metastasis. The administration of BRAF inhibitor, with or without MEK inhibitor, inhibits CD73 expression. It is becoming more widely recognized that there is a synergy when conventional antineoplastic medicines and CD73 blockage are used in conjunction to treat cancer. In mice with breast cancer that has spread to other parts of their bodies, CD73 suppression improves the effects of doxorubicin on the tumor. In contrast, CD73 blockade has a positive impact on the tumor microenvironment of irradiated tumors, as evidenced by an increase in CD8+ T cell infiltration and activity, a decrease in T-reg accumulation, and complete tumor regression when CD73 and CTLA-4 blockade are both used in conjunction with radiation. 2019 [Zhang B. et al]

3. Anti-CD73 in cancer immunotherapy

Multiple methods are employed by tumors to avoid immune monitoring. Adenosine production in the tumor microenvironment is one such mechanism that significantly reduces antitumor T cell responses. A membrane-bound nucleotidase called CD73 that is expressed by tumor cells, immunological suppressor subsets such T regulatory cells (T-regs) and myeloid-derived suppressor cells, as well as endothelial cells, is responsible for producing adenosine within the tumor. Recent research reveals that CD73 can be specifically inhibited to decrease cancer and metastasis while also boosting the effectiveness of T-cell-directed treatments. The effects of adenosine on dampening the antitumor response are discussed in this review, along with the arguments in favor of targeting CD73 in the treatment of cancer [Smyth, M.J. et al., 2012].

Conventional treatments such as surgery, radiation, or chemotherapy can be combined with cancer immunotherapy using endogenous or adoptively transplanted antitumor T cells. But a number of barriers prevent the development of efficient antitumor T cell immunity. The development of a permissive microenvironment and the activation of numerous immunosuppressive pathways by tumor cells may work in concert to thwart efficient immune responses. This review will examine the most recent experimental data supporting the idea that extracellular adenosine and the A2A adenosine receptor (A2AR) constitute a critical axis in tumor immune escape by showing that CD73 on tumor cells hampers antitumor T cell responses. Importantly, our results highlight a feasible and prospective method of treating cancer by targeting CD73 in addition to, or instead of, the A2AR [Zhang B., 2010].

Due to its crucial function in anti-tumor immunity, CD73 has lately come to light as a prospective target for new immunotherapy. In fact, in preclinical tumor animal models, CD73 inhibition with monoclonal antibodies (mAb) or small molecule inhibitors like APCP has shown anticancer effects. In early phase clinical studies, several anti-

CD73 mAbs and selective small molecule inhibitors are also being investigated. Notably, several treatments can boost CD73 expression and function. The feasibility of targeting CD73 in conjunction with chemotherapy, radiation therapy, and other immunotherapies is supported by this scientific evidence [Zhang B. et al, 2020].

1. Inhibition of the adenosinergic pathway

The therapeutic potential of targeting the CD73-A2AR axis in several cancer types has been stimulated by the pioneering work of the Sitkovsky group demonstrating the promise of A2AR inhibition for cancer immunotherapy. In various mouse tumor models, inhibition of both CD73 and A2AR demonstrated synergy in the anti-tumor response. Importantly, patients with renal cell carcinoma (RCC) who received the A2AR antagonist (ciforadenant) alone or in conjunction with an anti-PD-L1 antibodY exhibited a positive clinical response [Zhang B. et al., 2020]. Furthermore, high levels of adenosine gene signature expression prior to treatment were related with ciforadenant-mediated anticancer activity, suggesting that adenosine gene signature might function as a predictive biomarker for adenosine blockade. Given their different expression patterns and nonredundant functions, co-inhibition of CD73 and other adenosinergic components (such as A2BR and CD39) is also a viable alternative, even though blocking CD73 and A2AR is probably the most effective method to counteract tumor-driving adenosine effects. For instance, blocking the enzymatic activities of CD73 and CD39 has a higher inhibitory effect on the suppressive function provided by human MDSCs [Zhang B. et al, 2020]. Additionally, at suboptimal levels, the anti-CD39/CD73 mAb combination promoted T cell proliferation in both cancer patients and healthy donors. In mice models of experimental and spontaneous metastases, a recent study also shown a synergistic anti-metastatic effect combining anti-CD39 mAb and A2AR antagonists. The crucial significance of an eATP-P2X7-ASC-NALP3-inflammasome-IL-18 pathway as a mechanism of action was attributed to CD39 enzyme blockade-mediated anti-tumor impact, which was distinguished from other drugs targeting the adenosinergic system. However, it has been suggested that a non-canonical adenosinergic pathway headed by CD38 may support the immunosuppressive TME, particularly by acting as a mechanism for tumor cell escape from PD-1/PD-L1 inhibition. The suppression of A2AR and A2BR along with CD73 blockage greatly improved antitumor immunity in mouse CAF-rich tumors, and CD73 was elevated via the adenosine-A2BR circuit in cancer-associated fibroblasts (CAF) [Zhang B. et al., 2020].

2. Immune checkpoint blockade (ICB)

Combining anti-CD73 with anti-PD-L1 and/or anti-CTLA-4 therapy improved therapeutic effectiveness in a number of murine tumor models as compared to monotherapy, A2AR and ICB co-targeting also demonstrated therapeutic synergy. Many cancer patients do not respond (innate/primary resistance) or develop resistance after an initial response, despite the fact that ICB is successful in some cancer patients (acquired resistance). This may be because the TME has alternate immunosuppressive pathways or immunosuppressive pathways brought on by medication. This idea is supported by the discovery that melanoma patients who received PD-1 treatment had their CD73 level increased. Comprehensive immune profiling also showed that after anti-PD-1 therapy, a distinct CD73 macrophage population persisted in glioblastoma patients. In a mouse model of glioblastoma, CD73 loss increased the effectiveness of anti-PD-1 and anti-CTLA-4 [Zhang B. et al, 2020].

3. Adoptive cell therapy

Tumor regrowth was facilitated by T cell effector activity after transfer of tumor-specific T cells via CD73 knockdown on tumor cells. During ACT therapy, CD73 overexpression was also seen in melanoma patients. Additionally, in a mouse model of T-cell immunotherapy, CD73 was upregulated in recurrent melanomas, offering a potential basis for combining ACT therapy with CD73 inhibition. Similar to ACT, A2AR pathway suppression in T cells improved the effectiveness of chimeric antigen receptor (CAR)-T cell treatment. The

therapeutic effectiveness of modified CAR-NK cells against CD73+ tumors in human lung cancer xenograft models was also improved by targeting CD73 activity using anti-CD73 antibodies. Therefore, CD73 inhibition could prevent tumor growth in living organisms that is reliant on both innate and adaptive immunity of ACT. In 2020, [Zhang B. et al]

4. Agonistic immunotherapy

Activating immunological co-stimulatory molecules on T cells is an unexplored domain, much like blocking immune inhibitory molecules. We recently showed using preclinical models that tumor resistance to agonistic immunotherapy targeting 4-1BB, an inducible costimulatory molecule in the TNFR superfamily, was conferred by CD73 expression by T cells, whereas anti-4-1BB therapy preferred to mediate CD73-negative effector T cell response for tumor inhibition. Additionally, combining CD73 blockage with anti-GITR (another effective T cell costimulatory drug) also produced a synergistic antitumor effect. Based on this intriguing finding, we deduce that the clinical development of immunotherapeutic agonists targeting TNFR costimulatory receptors, such as 4-1BB, GITR, OX40, or CD40 in combination with CD73 and/or other adenosinergic signaling molecules, may be appealing. In fact, early phase clinical trials are making use of the combination of CD73/A2AR inhibition and anti-OX40 [Zhang B. et al, 2020].

5. Chemotherapy

It has been demonstrated that CD73 plays a role in multidrug resistance. For instance, doxorubicin resistance was associated with CD73, particularly in TNBC patients. Treatment with doxorubicin enhanced CD73 expression, which suppressed CD8+ T cells. There have also been reports of increased CD47+CD73+PD-L1+ cell frequency in TNBC cells following treatment with additional chemotherapeutic drugs such carboplatin, gemcitabine, and paclitaxel. It was discovered that CD73 expression was inversely correlated with sensitivity to a number of chemotherapy treatments by examining the susceptibility of NCI-60 cell lines to a panel of chemotherapeutic medicines. Additionally, platinum-resistant ovarian cancer cells had higher CD73 levels. Additionally, it was discovered that IL-6 produced by mesenchymal stem/stromal cells increased CD73 in nasopharyngeal cancer, promoting cisplatin resistance. After chemotherapy, CD73 overexpression appeared to be an effort to balance off too much ATP released by tumor cells that were dying as a result of the treatment. Additionally, P-glycoprotein, a drug efflux transporter, and ABC transporters appear to be downregulated by CD73-mediated adenosine signaling, which may contribute to drug resistance [Zhang B. et al, 2020].

6. Radiation therapy

Radiation therapy, in addition to directly killing cancer cells, can indirectly influence the immune system. For instance, radiation caused NK, T, and B cell apoptosis yet attracted and activated DCs. It is believed that the ratio of ATP to adenosine affects how differently radiation affects immunological control. It's interesting to note that increased CD73 enzymatic activity was found in radioactively damaged lung tissue and is thought to contribute to the serious side effect of pulmonary fibrosis. In example, anti-CD73 mAb therapy dramatically decreased radiation-induced lung fibrosis, indicating that CD73 inhibition may be a potential strategy for reducing lung side effects brought on by the treatment of thoracic cancers. Human breast cancer cells express CD73 more when exposed to radiation, and radiotherapy combined with CD73 suppression improved the antitumor response because antitumor T cell response was improved. However, it was discovered that pharmacological suppression or knocking down CD73 could restore the ability of T24 human bladder cancer cells to proliferate, decreasing their sensitivity to radiation [Zhang B. et al, 2020].

7. Targeted therapy

In a randomized phase III clinical trial examining the effectiveness of the anti-HER2/ErbB2 mAb trastuzumab, high levels of CD73 gene expression were observed to substantially correlate with poor result, suggesting a potential role for CD73 in conferring tumor resistance to targeted treatments. In fact, in immunocompetent mice models of HER2/ErbB2-driven breast cancer, anti-CD73 mAb therapy increased the effectiveness of anti-ErbB2 mAb. A2AR inhibitors or anti-EGFR therapy in combination with anti-CD73 mAb therapy is now being tested in clinical studies for non-small cell lung cancer. Similarly, despite the fact that CD73 expression was not a standalone predictive predictor in melanoma, more advanced clinical stage illness was linked to greater CD73 expression. It's interesting to note that while growth factors and activating MAPK mutations increased CD73 expression, BRAF and MEK inhibitors significantly decreased CD73 expression. BRAF-mutated melanoma in mice was more effectively treated by blocking adenosine signaling in addition to BRAF and MEK inhibition. Together, these investigations open new doors for developing targeted therapeutics that target CD73-mediated adenosine signaling and shed light on how CD73 is controlled during the course of cancer treatment [Zhang B. et al, 2020].

Through overwhelmingly strong anticancer immune responses, several escape mechanisms aid cancer progression. These mechanisms comprise numerous cellular and molecular elements that form a complicated network in the tumor microenvironment, promoting the growth of cancer cells and immune responses that are anti-cancer. It's interesting to note that in a number of malignancies, focusing on these escape mechanisms has proven to be an effective immunotherapeutic anticancer strategy. The production of adenosine has been identified as one of the most significant methods employed by cancer cells to outweigh immunological responses among the several immune escape mechanisms. As a result, it has been discovered that numerous cancer forms overexpress adenosine, which is linked to disease progression and a poor prognosis. Two cell surface-expressed molecules, CD39 and CD73, function to produce adenosine from adenosine triphosphate (ATP). Adenosine monophosphate (AMP), which is produced when CD39 breaks down ATP, is converted into immunosuppressive adenosine by CD73. In the vicinity of the tumor, ATP breakdown causes the immunological danger signal (ATP) to be depleted and an immunosuppressive factor (Adenosine) to be produced. About 90% of the homology between the mouse and rat CD73 sequence and the human sequence suggests that it is a highly conserved sequence. It is a 548 amino acid homodimer molecule having a carboxyl terminal AMP binding site. In addition to its GPI-anchored surface-expressed form, CD73 is also found in the plasma of cancer patients in a soluble form (sCD73). However, there is disagreement over its purpose, prevalence, and predictive significance. 2019 [Farhad Jadidi-Niaragh]

The expression of CD73 can be altered by a variety of conditions. Adenosine is overexpressed as a result of CD39 and CD73 being expressed more than usual in the tumor microenvironment. Hypoxia is not the only factor that can cause the expression of CD73; other factors that can do so include growth factor independent 1 (Gfi1), signal transducer and activator of transcription 3 (STAT3), Sp1, IL-6, interferon (IFN) type I, TGF-1, IL-1, TNF-, prostaglandin E2, Wnt signaling, and protein kinase C (PKC). Contrarily, the expression of CD73 is suppressed by several stimuli, including IL-12, IL-21, IL-4, and IFN- (Farhad Jadidi-Niaragh, 2019). Adenosine is a strong immunosuppressive compound that can effectively inhibit the growth and operation of a variety of immune cells, particularly T lymphocytes. It is intriguing that it can activate immunological suppressor cells including regulatory T cells (T-regs) and myeloid-derived cells (MDSCs). Adenosine can stimulate the growth, angiogenesis, and metastasis of cancer cells in addition to its immunosuppressive effects. Adenosine receptors, such as the A1, A2a, A2b, and A3 adenosine receptors, which are expressed on different cells, mediate each of these effects. These significant tumor-promoting and immunosuppressive activities made CD73 a promising immunotherapeutic target for cancer treatment. As a result, multiple studies that focused on CD73 in various cancer models had encouraging outcomes. The effectiveness of this approach in treating human tumors, however, is not well understood. Due to numerous unresolved difficulties on this subject, clarification is required for the successful application of this therapeutic method in human tumors. 2019 [Farhad Jadidi-Niaragh]

The high expression of CD73 on both cancerous and healthy cells is a concern with the CD73 targeting strategy. Several immune cells, such as T cells (both CD4+ and CD8+ T cells), T-regs, and B cells, can express CD73 in addition to cancer cells. Additionally, a number of nonimmune tissues and organs can express CD73. It should be emphasized that crucial factors include the enzymatic activity and intensity of the CD73 protein, in addition to the quantity of cells that express this molecule. Targeting CD73 is thought to result in the apparent capture of healthy cells that express CD73. Since both host normal and cancerous cells in the tumor microenvironment promote the generation of adenosine and thereby enhance cancer progression, I think that blocking CD73 in healthy (immune or nonimmune) cells not only is not harmful but also helps to maximize the impact of immunotherapy [Farhad Jadidi-Niaragh, 2019]. Although blocking CD73 will be associated with ameliorative effects on healthy cells in the tumor microenvironment, it is unknown whether a similar result will be observed for other non-malignant organs. As a result, CD73 is implicated in platelet aggregation, and mutations in this gene lead to calcification of the hands, feet, and arteries as well as cardiovascular disorders. In my opinion, it is crucial to concentrate and suppress CD73 only in the tumor microenvironment using novel drug delivery systems that can precisely deliver anticancer therapeutics to tumor sites through enhanced permeability and retention effect or active targeting in order to prevent blocking of CD73 in nontumor sites. Additional research is required to identify and create human medication delivery methods that are both safe and efficient. 2019 [Farhad Jadidi-Niaragh]

Although the majority of clinical studies and preclinical studies have shown that CD73 promotes tumor growth, the correlation of CD73 expression with favorable prognosis in some cancers, such as ovarian and breast cancers, has made it difficult to determine with precision whether CD73 targeting is effective in all stages of cancer. Therefore, I think that CD73 targeting's effectiveness tightly depends on the type of cancer and the stage of the disease, and that administering this therapeutic approach requires an assessment of CD73 expression and prognosis. However, even in tumors that lack CD73, CD73 expression on tumor-resident immune cells encourages CD73 blockage. Therefore, additional research into CD73 as the general tumor target or a tailored drug is required in future investigations. The findings of the ongoing clinical trials in this field should address this issue. 2019 [Farhad Jadidi-Niaragh]

As was already noted, in addition to CD73, both CD39 and adenosine receptors are involved in the production and signaling of adenosine; however, more study is needed to discover which of these two can be blocked more effectively to treat tumors. In preclinical research, it has been shown that blocking CD73 has a significant inhibitory effect on the growth of tumors. However, our team has demonstrated that in mice bearing tumors, suppression of A2aR effectively restores T cell anticancer responses. Therefore, it appears that the best method to counteract the tumor-stimulating effects of adenosine is to suppress CD73 and A2aR. Since adenosine can also be produced via the CD73-independent pathway, I think that blocking both CD73 and A2aR will have the greatest anticancer impact. Initial findings from our ongoing experiment, which have not yet been published, also suggest that combination reduction of CD73 and A2aR in tumor models is effective. 2019 [Farhad Jadidi-Niaragh]

4. *Therapeutic Implications for Autoimmune Disorders**Errore. Il segnalibro non è definito.*

The idea that ADO and enzymes involved in adenosinergic pathways for its production may represent attractive therapeutic targets for autoimmune and inflammatory illnesses is supported by a number of research [Morandi F. et al, 2018]. . In order for 2-(cyclohexyl methylthio) adenosine 5-monophosphate (chet-AMP), a phosphorylated ADORA2A agonist (prodrug) to become activated, it must first be present in the body. Flogel et al. examined this drug's effects in mice with CIA. Chet-AMP is converted to functional chet-ADO by CD73, which causes immunosuppressive and anti-inflammatory effects to be triggered by its administration. These results identified a potential new treatment target for RA patients: ADOR agonists. Similar findings were made in a another study, where the injection of an ADORA2A agonist to CD73 mice caused them to develop arthritis at a

rate comparable to that of wild-type mice. This study's findings supported the idea that A2A signaling plays a protective function in the prevention of CIA. ADORA3 agonists had similar outcomes; the mechanism underlying this positive anti-inflammatory action was linked to the inhibition of TNF-production [Morandi F. et al., 2018].

ADORA2a was reported by Ingwersen et al. as playing two roles in EAE. In fact, treatment with the ADORA2a-specific agonist before the commencement of the disease slowed down disease progression and tissue damage, whereas treatment with the same agonist after the onset of the disease accelerated disease progression. These findings suggested that ADORA2a protects and has anti-inflammatory effects on T cells early on, but that as the inflammation in the CNS progresses, activation of this receptor may cause tissue damage. In EAE mice given an ADORA2a agonist, Liu et al. noticed a restriction in the disease's course that was caused by a decline in T cell proliferation, CNS infiltration, and cytokine production. Caffeine treatment for EAE mice enhanced the expression of ADORA1 on microglia, which led to a subsequent decrease in the severity of the EAE, as shown by Tsutsui et al. Concurrent use of the ADORA1 agonist furthered this effect, indicating that ADORA1 may serve as a therapeutic target to control neuroinflammation in MS and other demyelinating illnesses. 2018 [Morandi F. et al]

ADO receptor agonists have also been demonstrated to have therapeutic effects in other autoimmune disease preclinical models. A nonselective ADO receptor agonist in diabetic mice delayed the onset of diabetes in animals given MLDS or cyclophosphamide. It is possible that only ADORA2b offers a promising therapeutic target for type 1 diabetes given that this effect may be reversed with ADORA2b antagonists but not ADORA1 or ADORA2a antagonists. A prospective therapeutic target for myasthenia gravis is ADORA2a. In fact, it has been shown that activating ADORA2a with a particular agonist lessened the growth of acetylcholine receptor-specific autoreactive lymphocytes and the production of anti-AChR antibodies while also lessening the severity of the disease in rats with experimental autoimmune myasthenia gravis (EAMG). 2018 [Morandi F. et al] ADO, ADO receptors, and other similar molecules are the subject of a small number of active clinical trials in individuals with autoimmune/inflammatory illnesses. Phase I, phase IIa, and phase IIb human investigations have all been conducted using CF-101. Patients with RA experienced an anti-inflammatory and anti-rheumatic response as a result of the medication, which was well-tolerated and safe. ADORA3 may serve as a biologic marker to anticipate a patient's reaction to the medicine, as evidenced by the substantial association between ADORA3 expression level and response to drug reported [Morandi F. et al, 2018].

5. Potential treatment of Renal Ischemia with 5’-Nucleotidase

The synthesis of extracellular adenosine plays a crucial role in IP's ability to protect the kidneys. Preconditioned renal tissue's transcriptome showed that CD73 was strongly induced. Similar to this, CD73 mice can reduce increases in renal adenosine levels caused by IP. The renal protective effects of in situ IP were eliminated by pharmacological suppression or targeted gene deletion of CD73. Additionally, soluble 5’-nucleotidase therapy mirrored IP's ability to protect the kidneys by increasing renal resistance to ischemia to a similar degree as IP-exposed mice. All of these investigations point to increasing extracellular adenosine concentrations by manipulating CD73 enzyme activity as a potential therapy for acute ischemia kidney injury [Grenz A. et al., 2007].

It is still unclear where exactly extracellular adenosine produced in hypoxic or ischemic circumstances comes from. When there is inflammation or hypoxia, polymorphonuclear leukocytes, platelets, or endothelia can release ATP. Extracellular ATP has the potential to either communicate with renal ATP receptors directly or serve as a metabolic substrate for ATP/ADP to AMP conversion to adenosine via CD39 (ecto-apyrase) and ecto-5’-nucleotidase. It follows that CD39 may also have a role in renal protection during ischemia in addition to CD73. According to Grenz A. et al. (2007), CD39 can affect extracellular adenosine production in two different ways:

1) Extracellular ATP/ADP is converted to AMP by CD39 in order to supply the metabolic substrate for CD73-dependent adenosine synthesis.

2) CD39 is in charge of lowering extracellular ADP levels, which removes ADP as a CD73 feed-forward inhibitor.

According to earlier research, intestinal epithelia or endothelia exposed to ambient hypoxia (2 percent oxygen) experienced a significant induction of CD73 transcript, protein, and function. This finding is supported by the observation that the induction of renal CD73 with IP is also consistent with this finding. Similar experiments in CD73 animals showed increased pulmonary edema and vascular leak syndrome after exposure to hypoxia, supporting a role for CD73-dependent adenosine synthesis in maintaining the function of the vascular barrier under hypoxia. Therefore, it is tempting to hypothesize that HIF-1 regulates transcription, resulting in the observed renal protective effects of CD73-dependent adenosine synthesis during renal IP. Additionally, hypoxic preconditioning causes the kidneys to produce erythropoietin in an HIF-1-dependent manner, protecting the heart against ischemia. A. Grenz et al., 2007.

6. Targeting the Immunomodulatory CD73/Adenosine System to Enhance Radiation Therapy Therapeutic Gain

The lung, among other normal organs, depends on extracellular adenosine, a potent endogenous immunosuppressive mediator, to maintain homeostasis. The ectoenzymes ectoapyrase (CD39) and 5' ectonucleotidase (CD73), which catabolize ATP to adenosine, work in concert to either release adenosine from stressed or wounded cells or produce it from external adenine nucleotides. While chronically elevated adenosine levels in tissues exposed to DNA-damaging chemotherapy or radiotherapy promote pathologic remodeling processes and fibrosis, for example in the skin and lung, an acute CD73-dependent increase in adenosine in normal tissues primarily exerts tissue protective functions. Importantly, CD73 is expressed by cancer cells as well, and high levels of CD73 expression in tumor tissue have been associated with poor overall survival and recurrence-free survival in patients with breast and ovarian cancer. Cancer cells' growth-promoting neovascularization, metastasis, and survival are supported by CD73 and adenosine. Adenosine can also facilitate tumor intrinsic immune escape or immunological escape brought on by therapy through a variety of immune system suppression pathways. In order to stop tumor growth, enhance anticancer immune responses, prevent therapy-induced immunological deviation, and maybe reduce normal tissue toxicity, regulating CD73 or cancer-derived adenosine in the tumor microenvironment emerges as an alluring novel therapeutic option. Less is known about the function of CD73/adenosine signaling in the tumor and normal tissue responses to radiotherapy, as well as its usage as a therapeutic target to enhance radiotherapy treatments. With a particular emphasis on the lung, the current review will highlight the dual function of CD73 and adenosine in tumor and tissue responses to radiation. In order to maximize the therapeutic value of radiation or combination radioimmunotherapy for the treatment of cancer, it will also cover the possible advantages and disadvantages of pharmacologic modulation of the CD73/adenosine system [Jendrossek V., 2019].

The development of cancer immunotherapy and the knowledge that radiotherapy, particularly when combined with immune checkpoint blockade, activates T-cell-mediated antitumor immune responses under specific circumstances sped up interest in and research into the potential advantages of combining radiotherapy and immunotherapy in pre-clinical and clinical cancer research. Determining the best dosage and treatment plans and comprehending the dual aspects of radiation-induced immunity alterations that may have an impact on unfavorable immune-related outcomes remain significant issues. Additionally, due to the possibility that tumors lack immunogenicity, possess effective means of evading tumor immune surveillance, or that responses are not long-lasting, only a small percentage of patients respond to treatment with immune checkpoint blockade alone or in conjunction with radiotherapy [Jendrossek V., 2019].

One typical method by which cancers evade tumor immune surveillance is the accumulation of extracellular adenosine by activation of 5' ectonucleotidase (CD73) and subsequent signaling through adenosine receptors. Due to this, CD73/adenosine signaling is a desirable immuno-oncology target, and numerous reviews have a thorough discussion of the relevant research and guiding ideas [Jendrossek V., 2019].

Less is known about the function of CD73/adenosine signaling in tumor and normal tissue response to radiotherapy, as well as its potential influence on radiotherapy and combined radioimmunotherapy outcomes. It is crucial to keep in mind that between acute and chronic activation stages as well as across tissues of various sources, the effects of CD73/adenosine activation on the immune system and the restoration of tissue homeostasis may vary. In order to better understand how radiotherapy-induced alterations in innate and adaptive immune cell compartments contribute to both the positive and negative effects of radiotherapy, we will first discuss how these changes affect both acute and chronic tumor and normal tissue responses. The function of CD73 and adenosine in tumor and normal tissue responses to radiotherapy has been established, and the potential of targeting CD73/adenosine for enhancing the therapeutic gain of radio-immunotherapy in thorax-associated tumors with high risk of adverse late effects in the highly radiosensitive normal lung tissue has also been demonstrated [Jendrossek V., 2019].

Ionizing radiation exposure has the potential to trigger immunological responses in both normal and malignant tissues, as was mentioned above. Cells from the irradiated cancer or healthy normal tissues interact intricately with cells from the innate and adaptive immune systems to cause these alterations. However, based on the immune status of the tissue prior to exposure to ionizing radiation (pro- vs. anti-inflammatory) and the type (tumor vs. normal) and origin of the irradiated tissue, as well as the temporal appearance (acute vs. chronic), the immune response can either adopt immunostimulatory or immunosuppressive effects and have either a positive (anti-tumor; normal tissue protection) or a negative (pro-tumor, normal tissue toxicity) about the results of the treatment [Jendrossek V., 2019]. Targeting tumor-induced or radiation-induced immune deviation may present novel and appealing opportunities for improving the outcome of radiotherapy by modulating the tumor radiation response, radiation-induced adverse late effects, or both, according to a number of findings from pre-clinical and clinical studies summarized in the previous paragraphs. However, the complexity of the tumor- and radiation-induced changes in the microenvironment as well as the time- and tissue-dependent "dual face" of radiotherapy-induced immune changes highlight the significance of developing strategies that balance the negative pro-inflammatory and immunosuppressive effects of radiotherapy and outweigh the positive effects of radioimmunotherapy with the goal of achieving the best tumor control and normal tissue protection. Research has recently turned its attention to the purinergic CD73/adenosine system in this context because it is a crucial endogenous regulator of the innate and adaptive immune systems and has been linked to both tumor immune escape and the unfavorable late effects of radiotherapy [Jendrossek V., 2019].

The purinergic system might present brand-new chances to obstruct the way radiation therapy and radiation-induced immune variation affect regular tissue and tumor responses. Extracellular ATP, one of the aforementioned DAMPs that serve as immunostimulatory pro-inflammatory signalS, is a danger signal generated by dying and injured cells. Extracellular adenosine, on the other hand, primarily performs anti-inflammatory, immunosuppressive, or regulatory functions. It is a key mediator for the preservation of tissue homeostasis in a variety of tissues, including the lung, as well as for preventing excessive inflammation, such as that brought on by an infection. However, maintaining or reestablishing immunological homeostasis as well as orchestrating tissue inflammation and repair under conditions of damage-induced sterile inflammation may depend on maintaining a balance between pro-inflammatory ATP and anti-inflammatory adenosine [Jendrossek V., 2019].

7. Current Research and Future Perspectives

CD73/adenosine signaling is a unique pathway that both locally and systemically promotes RILD. The aggregation and/or alternative activation of macrophages in organized clusters, their production of pro-fibrotic mediators, or both, were consequences of pathologic CD73/adenosine signaling. In the irradiated lung environment, the radiation-induced rise in CD73/adenosine is required to amplify pro-fibrotic signaling by fostering the complex interactions among damaged resident cells, infiltrating immune cells, immunosuppressive T-reg, and other pro-fibrotic mediators like hyaluronic acid and TGF-. 2019 [Jendrossek V.]

The tissue-specific effector and target cells of CD73/adenosine-signaling in response to genotoxic treatment (BLM, radiotherapy) are still debatable and require further research, despite immunomodulatory effects of adenosine having been linked to CD73/adenosine-induced adverse effects in other injury models. In this respect, endothelial cell dysfunction and loss as a long-term complication of radiation-induced normal tissue toxicity have also been connected. WTI increased total CD45+ leukocyte counts, notably profibrotic CD11b+ myeloid cells and Ly6C+ inflammatory monocytes, in the lungs of radioirradiated mice as a direct result of decreased vascular function. On the other hand, over time, endothelial loss, thickening of the basement membrane, persistence of activated pro-coagulant endothelial cell type, and collapse of microvessels will all help to create a hypoxic, pro-inflammatory illness state. The pathologic environment includes resident cells and immune cells that have increased levels of ADOR and CD73 due to hypoxia. Although it is tempting to assume that therapeutic suppression of CD73 would similarly influence negative late consequences in the lung by lowering radiation-induced vascular damage, this is yet to be proven. It's interesting to note that subsequent research shows that locally irradiated MSC contribute to the pathogenesis of radiotherapy-induced pulmonary fibrosis by developing a pro-fibrotic myofibroblast-like phenotype that encourages extracellular matrix deposition, tissue remodeling, and pulmonary fibrosis upon WTI. Future research should examine if the expression of CD73 on the surface of endothelial cells or resident MSC affects the development of RILD because it is expressed on endothelial cells and MSC of healthy lungs. The same is true for CD39 and CD73 expression on cancer exosomes, which have similarly been demonstrated to inhibit T cells by producing adenosine. 2019 [Jendrossek V.]

Adenosine affects the tumor microenvironment and restricts tumor immunity on a number of levels when it is secreted in an inflammatory environment or produced via the CD39/CD73 axis. In order to slow tumor growth and enhance antitumor immune responses, modulating cancer-derived adenosine in the tumor microenvironment appears to be a promising strategy. Furthermore, our own research suggests that this may be feasible without significantly raising the risk of late complications involving normal tissue. Thankfully, several methods for pharmacologically altering adenosine levels already exist or are in development, and numerous clinical studies have been started to assess the use of novel inhibitors of CD73 or ADORA2A signaling in cancer therapy both alone and in conjunction with immune checkpoint blockade. These research will provide information on effectiveness, compatibility, and any negative effects. 2019 [Jendrossek V.]

ADORA2A has received a lot of attention in cancer since it is well recognized to effectively reduce immune responses in malignancies and healthy tissues. However, it must be remembered that other ADOR may be more significant depending on the tissue of origin and the molecular and immunological signature of the tumor. Furthermore, it is still completely unknown how purinergic signaling affects how cancerous tumors respond to radiation therapy and whether CD73 or ADOR inhibitors can improve the effectiveness of RT both on its own and in combination with immunotherapy. Finally, there are currently no trustworthy biomarkers for the diagnosis or prediction of a patient's likelihood of developing RILD after treatment. Therefore, more research is required to link the result of radio(chemo)therapy or immunotherapy to the gene and protein expression of CD73 and the ADORAs. Extracellular adenosine levels will fluctuate based on the tissue, the treatment mechanism, and intensity in a spatiotemporal manner. As previously established, the receptors have different affinities for adenosine. Therefore, it would be very helpful to conduct an immunoscore on tissues from pre-clinical trials and examine the correlation between CD73 and the ADORAs expression levels and the existence of immunosuppressive lymphoid and myeloid cell subsets, as well as possible tissue hypoxia. Such information might then be used to patient samples. An important finding in this case was that whereas high CD73 expression

in the tumor stroma was suggestive of a prolonged recurrence-free survival, high CD73 expression in normal tissues was indicative of a poor infiltration of prostate tumors with CD8+ T cells. This emphasizes the need to assess CD73 expression in both normal and malignant tissue [Jendrossek V., 2019].

Multiple degrees of CD73 activity restrict antitumor effects. A fascinatingly multifaceted way that adenosine produced by both the tumor and the host CD73 affects cellular antitumor immune responses is by inhibiting the activation, clonal expansion, and homing of tumor-specific T cells with helper and cytolytic effector function, reducing the survival of CTL, changing T-reg activity, and enhancing the conversion of type 1 macrophages into tumor-promoting type 2 macrophages. The engagement of CD73 as a proliferative factor, being involved in the regulation of cell growth, differentiation, invasion, migration, and metastatic processes has been shown, in addition to the immunoregulatory functions of CD73. Additionally, adenosine produced by CD73 can favorably influence the adhesion and/or chemotaxis of tumor cells. The possibility that CD73 might aid tumor angiogenesis is also fascinating. The primary proangiogenic effects of adenosine can be attributed to its capacity to promote endothelial cell migration and proliferation as well as to modulate the vascular cells' and immune cells' ability to produce proangiogenic molecules like vascular endothelial growth factor in hypoxic tumor tissues. The actual in vivo effects of a therapeutic strategy that uses CD73 as a molecular target for cancer treatment may significantly outperform the expectations raised by our published experimental data given the immune and non-immune actions of CD73-generated adenosine on the tumor microenvironment [Zhang B., 2012].

From a translational standpoint, CD73 small molecule inhibitors and monoclonal antibodies are readily available, safe for mice, and have already been utilized in vivo to treat cancer in a number of mouse tumor models. Thus, CD73 blockade's progress and potential clinical uses are highly positive. It's crucial to remember nevertheless that blocking CD73 on its own is ineffective in treating cancer. Adoptive T-cell therapy and dendritic cell vaccines are examples of other strategies that should be used in conjunction with countering the immunosuppressive adenosinergic effects of CD73 in the tumor microenvironment in order to obtain the best anti-tumor strategy [Zhang B., 2012].

It should be noted that targeted CD73 therapy has broader clinical implications than just certain classes of solid cancers. In circumstances when cancer cells themselves lack or lose CD73 expression, the host CD73 becomes a possible therapeutic target for slowing tumor growth [Zhang B., 2012].

In both tumor and host cells, CD73 plays specific functions in the regulation of antitumor immunity. In actuality, all 4 adenosine receptor subtypes have a role in regulating tumor development. It now appears that CD73-mediated adenosinergic effects, working in a coordinated manner involving indirect and direct actions on various cellular components like cancer cells, endothelial cells, and immune cells, have a significant impact on tumor growth and metastasis. This is in line with earlier research by others. These affirm the viability of effective methods that target the crucial tumor axis of CD39/CD73-adenosine receptors (ARs) in order to harness anticancer immune responses. The idea has been brought up that therapeutic targeting of ARs may cause unintentional tissue injury and/or aberrant tissue remodeling. Based on the individual illness etiology and the degree of inflammation, additional caution may be needed in the timing and strength of anti-adenosinergic treatment. 2012 [Zhang B.]

Additionally, an anti-CD73 mAb's effects can go beyond only inhibiting CD73's enzymatic activity. For instance, anti-CD73 mAb may specifically prevent tumor cells from adhering to endothelial cells, hence reducing their capacity for invasion. Be aware that the anticancer effects of APCP or anti-CD73 mAb described above may or may not be solely attributable to the suppression of CD73 enzyme activity on tumor cells. It seems that CD73's production of extracellular adenosine can defend tumors both tumor-autonomously and by preventing host T-reg from suppressing entering antitumor T cells. In fact, it was discovered that CD73 is overexpressed on T-reg cells and that the combination of CD39 and CD73 inhibits T cell activity. There is evidence that the host CD73 plays a crucial role in the T-reg immunosuppressive system and the endothelium barrier that prevents antitumor

T cells from homing to malignancies (submitted). Accordingly, the real in vivo effects of a therapeutic strategy that exploits CD73 as a molecular target for treating cancer may greatly surpass the expectations set by our reported experimental data. [B. Zhang, 2010]

Opportunities to create efficient and targeted immunotherapies for different types of human cancers have been presented by the developing identification of the roles of CD73 in tumor growth and metastasis. 2012 [Zhang B.]

Because CD73 is expressed on numerous host cell types, including subsets of lymphocytes, endothelial cells, and dendritic cells, Stagg and his colleagues later studies using the same CD73-deficient mice focused on the crucial role of host CD73 expression and activity in a number of transplantable tumor models. Collectively, these investigations showed that:

- ☐ Immunogenic tumor development and metastasis are inhibited in CD73-deficient mice;
- ☐ CD8+ T cells are required for the protective effect of the host's CD73 deficit on initial tumors, and this effect is linked to elevated endogenous antitumor T-cell immunity;
- ☐ Both hematopoietic and nonhematopoietic cells must be CD73-deficient in order to prevent increased tumor antigen-specific T-cell homing to tumors and control tumor growth.

According to prior research, CD4+CD25+ regulatory T cells' protumorigenic activity in the hematopoietic compartment was partially reliant on their expression of CD73. On the other hand, CD73 expression on nonhematopoietic cells is necessary for the prometastatic action of host-derived CD73 [Zhang B., 2012]. Established carcinogen-induced tumors had their growth effectively slowed by anti-CD73 mAb therapy. CD73 has a crucial role in tumor growth and metastasis from both the tumor and the host [Zhang B., 2012]

Further complicating matters, CD73-derived adenosine can directly promote tumor angiogenesis, tumor cell proliferation, invasion, migration and adhesion, and/or chemotaxis in addition to the immunoregulatory functions of CD73. As a result, the real in vivo effect of targeted CD73 cancer treatment may be far more than what was anticipated based on published experimental findings [Zhang B., 2012].

What is the genuine therapeutic potential for targeted CD73 cancer therapy, given the existing accessibility of CD73 blockage for anticancer treatment for preclinical studies? In certain mouse tumor models, small-molecule inhibitors and mAb against CD73 are efficient cancer treatments that are also well tolerated in mice. Furthermore, independent studies have supported the significance of the CD73-mediated adenosinergic pathway in the expansion and invasion of human cancer cells as well as T-reg-induced immunosuppression in a variety of human malignancies. Therefore, the creation of CD73 blockage using small molecule inhibitors and clinical-grade antihuman CD73 mAb is required. Despite these encouraging findings, the majority of published trials to date have limitations about the size and time of tumor growth prior to anti-CD73 therapy. Although these studies have provided compelling experimental support for the possible use of CD73 inhibition in the treatment of small tumors, the immunological state of an early-stage tumor and a big established tumor differs significantly. This issue is more evident in the clinical situation because patients have existing malignancies and immune systems that are already dysregulated. In fact, blocking CD73 alone is ineffective in treating cancer. Furthermore, when big established tumors were treated with CD73 inhibition, fewer therapeutic effects were seen. However, the heterogeneity of cancer would likely prevent any particular cancer immunotherapy from serving as an example of effective treatment. 2012 [Zhang B.]

In light of these difficulties, researchers have been motivated to investigate the potential for developing the most effective anticancer method using CD73 targeting. Last but not least, tumor CD39/CD73 has the capacity to convert extracellular ATP released from tumor cells induced by chemotherapeutic drugs and radiation into

adenosine, rendering tumors more resistant to immune-mediated killing or drug-induced death [Zhang B., 2012]. This means that CD73 blockade could be successfully combined with chemotherapy or radiation therapy.

Although CD73 has been found in a variety of cancer forms, including leukemia, its clinical significance has not yet been fully established. Studies show that CD73 expression may be a diagnostic/prognostic marker and/or therapeutic target in some cancer types. It is likely linked to more aggressive, metastatic tendencies. Nevertheless, contradictory data have suggested that, depending on the kind of human cancer, CD73 expression was associated with both a bad and a good clinical outcome. Therefore, it would be intriguing to revisit this area and investigate any potential correlations between CD73 and the clinical outcome in a large cohort of various cancer specimens and the phenotypic characterization of various immune infiltrates [Zhang B., 2012]. Although CD73 expression in tumors has sparked renewed interest, we still know very little about it and have a lot to learn. For instance, what factors influence CD73 expression in the tumor microenvironment? Due to the unknown factors influencing CD73 expression in each specific situation during cancer growth, the in vivo picture is probably far more complicated. Factors that promote CD73 expression are present in the tumor microenvironment (Zhang B., 2012).

Anti-CD73 therapy has not yet been linked to any on-target negative side effects, however it has been shown to lessen tumor burden in mice models. The potential hazardous danger linked to CD73 blockage is still being investigated, though. A variety of inflammatory responses in CD73-deficient animals have been highlighted as being aggravated. Furthermore, ectopic tissue calcification that is linked to an elevated risk of cardiovascular events is accelerated by human CD73 nonfunctional mutations. This has not been seen in CD73-deficient mice, suggesting that in some contexts, the expression and/or function of the mouse homolog of CD73 may be different from that of humans. Therefore, when future research aims to translate this therapeutic approach to clinical trials in cancer patients, additional caution may be needed in the timing and intensity of anti-CD73 treatment based on the individual disease etiology and the stage of inflammation. 2012 [Zhang B.]

Inhibiting CD73 activity may improve anti-tumor immune surveillance at the level of T cells and other immune cells regulated by adenosine, according to preclinical research that has linked CD73 to immunological escape in cancer. One important tactic to aid in triggering an anti-tumor immune response may be to target the CD73 pathway in conjunction with other possibly complimentary immunological pathways 2012 [Zhang B.].

In addition to its immune-dependent actions, CD73 also encourages carcinogenesis without also having an immune-dependent effect. It is anticipated that CD73 blockade will work in conjunction or synergy with other immune checkpoint inhibitors and agonist immunotherapies. Further testing is necessary to determine whether CD73 on the surface or in solution is a useful biomarker [Zhang B. et al., 2019]. In the context of individualized cancer therapy, there is a relationship between CD73 and the initiation, development, and spread of malignancies, underlining the potential relevance of this protein as a target and as a novel biomarker [Antonioli L. et al, 2016].

Cancer immunotherapy is a fast developing discipline, and CD73-targeted treatments are a useful illustration of a novel immune target resulting from a fundamentally novel therapeutic strategy. Despite the efficient anticancer effects seen in preclinical studies when CD73 inhibition is combined with the blocking of other immune checkpoints or agonist immunotherapies, the evident therapeutic potential of CD73-targeted therapy in cancer patients has only recently been characterized. Additionally, it seems intriguing to manipulate CD73's role in adenosine metabolism as a therapeutic strategy for the downstream cascade (such adenosine deaminase). In-depth analysis of CD73 expression, its role, and the subsequent cascade in the tumor microenvironment during ongoing clinical trials will be crucial to determining whether or not inhibiting the CD73-mediated adenosinergic axis will significantly help people with aggressive cancer. 2019 [Zhang B. et al]

FINAL REMARKS

This thesis provides evidence that CD73 has a role in various clinical applications and functions in diseases affecting the whole body system; involving the Lymphatic system, Urinary system; Endocrine system, Reproductive system, Respiratory system, Digestive system, Cardiovascular system, Nervous system and Integumentary system.

Reduced adenosine synthesis may alter the synovial milieu, inhibiting the control of chronic inflammation, as suggested by the significantly lower number of CD73+ synovial cells in patients with the more severe subtype of the condition. Furthermore, cells that are growing and shedding their CD73 may be the source of CD73 downregulation. Future research is required to specify how precisely this altered expression of an immune-regulatory molecule fits into the larger context of arthritis etiology. In the future, it is likely that medications may modulate the adenosine-generating pathway, and if they do, inflammatory arthritis may be a category of disorders where they are investigated for a potential therapeutic benefit (Jacobson et al. 2006; Gessi et al. 2011).

The potential of CD39/CD73/adenosine-signaling as a prospective therapeutic target in immuno-oncology is highlighted by a number of pre-clinical studies when taken collectively. The activation of T-cell dependent tumor immunity has so far been linked in numerous studies with the effects that have been seen. In the tumor microenvironment, it's crucial to take into account CD73's and adenosine's additional immunoregulatory effects, specifically how they may affect the biology of macrophages and myeloid cells, respectively [Jendrossek V., 2019]. Adenosine produced by CD73 inhibits antitumor immunity and promotes the growth and/or spread of tumors. In addition to its immune-dependent actions, CD73 also encourages carcinogenesis without also having an immune-dependent effect. Surprisingly, tumor angiogenesis is suppressed by CD73 inhibition, and CD73-mediated enzyme and nonenzyme functions both promote tumor angiogenesis. Additionally, CD73 encourages extracellular matrix proteins and tumor cell attachment. These findings have maintained interest in the quick advancement of anti-CD73 cancer therapy employing monoclonal antibodies (mAb) and/or small molecule inhibitors in preliminary clinical trials. Furthermore, poor prognoses and lymph node metastases are linked to elevated CD73 levels in a number of cancer types. Prostate cancer and triple-negative breast cancer patients have been discovered to have CD73 as an independent predictive factor (TNBC). Additionally, a recent study contends that sCD73 is a trustworthy biomarker in people with metastatic melanoma who are taking nivolumab. Further research is necessary to determine whether surface CD73 and/or sCD73 are useful biomarkers [Zhang B. et al., 2019]. To stop anti-tumor immune surveillance at the level of T and natural killer (NK) cells, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and tumor associated macrophages (TAMs), CD73 converts extracellular adenosine monophosphate (AMP) into immunosuppressive adenosine [Kobie JJ, 2006]. According to the expression of CD73 by Th cells and the enrichment of both enzymes in murine T-reg cells, Th cells are involved in the local regulation of inflammation and adenosine buildup. Reduced levels of adenosine synthesis in APCP-treated cells or mice lacking the CD73 gene were linked to an improved pro-inflammatory response that inhibited Salmonella colonization after exposure. These findings lend credence to the idea that adenosine synthesis and its capacity to reduce inflammation encourage bacterial survival, extending the scope of infection. According to certain research, CD73 expression may be linked to tumor promotion, which strengthens the ability of cancer cells to spread across the body. This demonstrates that CD73 has a role in the metastasis of malignant melanoma, gastric cancer, and prostate cancer [Ranjbar M-A. et al., 2019]. Numerous studies have out the importance of carefully weighing the cardioprotective effects of CD73 when systemic anti-CD73 therapy is being explored for conditions other than cardiovascular ones, like cancer [Minor M. et al, 2019]. Low CD73 expression in stromal cells and high CD73 expression in cancer cells is preferable, but high CD73 expression in stromal cells and low CD73 expression in cancer cells is associated with poor overall survival. 2020 [Harvey J.B.]

In CRC, CD73 is a biomarker that predicts how well patients will respond to anti-EGFR therapy. 2020 Harvey J.B. . Studying the illness causes and outcomes of people with inherited CD73 deficiency will help us better understand what patients receiving anti-CD73 monoclonal antibodies, and so partially acquiring CD73 deficiency, may suffer. CD73 is becoming a significant target in cancer therapy. Given that these antibodies may increase the risk of vessel matrix dysregulation, joint calcifications and pain, heart failure, and ectopic medial calcification, long-term monitoring of the effects of receiving these antibodies is essential. Those who have co-morbidities, such as diabetes mellitus, chronic kidney disease, osteoporosis, or hypertension, are especially at risk for this type of calcification. According to Joolharzadeh P. et al. (2019), this novel and fascinating population undergoing experimental therapy could also serve as another model system. During an acute T. gondii infection, adenosine synthesis is hindered, which is similar to the intestinal immunopathology brought on by dysbiosis and intestinal barrier disruption. Adenosine may make an intriguing target for immune intervention in the case of inflammatory bowel diseases, according to research by Oldenhove G. et al. (2015). Activation of the adenosine A2A and A2B receptors not only attenuated tissue damage and downregulated inflammatory responses, but it also decreased parasitic load. Intestinal epithelial cells' expression of CD73, a crucial enzyme in the creation of extracellular adenosine, is a crucial regulator of Salmonella's intestinal colonization and pathogenicity and has a sizable impact on the spread of germs to extraintestinal locations. These findings point to adenosine as a possible therapeutic target and as an endogenous regulator of the gut microbiota. The mechanism by which adenosine exerts antibiotic effects and modulates Salmonella virulence is still being studied [Kominsky D.J. et al, 2017]. Due to their high level of activation, CD8+ T cells express far less CD73 during HIV infection. Due to the loss of CD73's coactivator function, this drop is likely to be involved in the decreased ability of HIV-specific CD8+ T cells to multiply. Consequently, it is likely that an upregulation of CD73+CD8+ T cells plays a role in the inhibition of viral replication in HICs by the CD8+ T-cell immune response [Carrière M., et al., 2014].

The purinergic signaling system is essential for many physiological processes, and CD73 is one of its main regulators. But its significance becomes particularly clear in the setting of acute and chronic damage types, where CD73 activity is essential for preserving tissue integrity and promoting recovery. The in vivo functions of CD73 that are particular to different cell types will be studied during the coming years using tissue-specific deletion mice models together with ex vivo primary cultures. It will also be critical to identify which traits in the animal models are representative of the patients in humans. The latter will entail creating fresh resources for studying CD73's function, namely tissue organoids and human iPSC-derived cells. It is also unclear if systemic CD73-based therapy is possible or whether more focused methods would be required to prevent adverse effects. While there is a wealth of preclinical data supporting the development of CD73-based therapeutics, it will be crucial to comprehend how these could affect regular physiological functioning [Minor M. et al, 2019].

Some significant broad implications to consider amongst other:

1) It's critical to look at unconventional adenosine production mechanisms; and
2) When a specific pathway in complex biological systems is blocked by genetic or pharmacological means, other pathways are frequently activated.

There number of practical applications to consider:

1) Researchers should be mindful that the absence of CD73 inhibition's effects does not preclude the possibility that adenosine or adenosine receptors play a role;
2) TNAP should be a target in the development of therapeutic methods to control adenosine synthesis; and
3) Increasing the local generation of tissue-protective adenosine could be medically accomplished by alkaline phosphatase itself. Alkaline phosphatase is in fact being researched to prevent acute renal failure in sepsis patients, with good early results.

Human breast cancer cells are stimulated to proliferate by CD73 via the AKT/GSK-3/-catenin/cyclinD1 signaling pathway. In light of this, CD73 may one day serve as a significant clinical and prognostic biomarker for breast cancer [Zhou P. et al, 2017]. Strong evidence suggests that CD73-mediated immunosuppression reduces anthracycline efficacy, which may be a factor in certain TNBC patients' worse outcomes, particularly in those who had high levels at diagnosis (pretreatment). Despite the fact that CD73 inhibition and anthracyclines appear to work in concert, we have not conclusively established that this effect is unique to anthracyclines, and it is entirely possible that this effect might also occur with other chemotherapeutic drugs. Anthracyclines, however, are frequently the go-to treatment for these patients. Interestingly, we found that chemotherapy using the regularly used taxane PAC did not benefit from anti-CD73 therapy. In light of all of the available information, we hypothesize that CD73 and its downstream effector A2A adenosine receptor might serve as special therapeutic targets in this subgroup of breast cancer patients who are currently characterized by a relative lack of targeted treatment options [Loi S. et al, 2013]. A new modulator of CB chemoafferent action is CD73. Inhibition of CD73 dampens hypoxia-induced responses and lowers basal CB sensory neuronal activity. In vivo CD73 inhibition modulates circulatory alterations in hypoxia, including decreased HR rise, and blunts HVR. Future research will be crucial to determine whether CD73 is a viable target for lowering sympathetic outflow and CB activity in CB-related cardiovascular disease [Holmes A.P. et al, 2017]. Immune systems are trained to spot cancer cells through cancer vaccinations. In this situation, focusing on A2AR is also a promising tactic. A2AR-deficient animals demonstrated greater proliferation of tumor-specific CD8+ T cells and enhanced survival as compared to wild-type mice, increasing their responses to melanoma and lymphoma tumor vaccines. Genetic CD73 or A2AR deletion or inhibition also improves the efficacy of adoptive T cell transfer. Due to greater infiltration and activation of adoptive T lymphocytes, tumor-bearing mice experience improved tumor control and survival [Harvey J.B., 2020]. According to this research, CD73 produces extracellular adenosine, which is a crucial regulator of arteriogenesis and consequently affects the arterial remodeling process. Monitoring the dynamic changes in vascular blood flow following artery ligation and HEP in the afflicted muscle was made possible using MRI and MRS. The effect of this nucleoside on monocyte transendothelial migration is most likely the mechanism of action of adenosine. Changes in the phenotype of monocytes caused by adenosine that resulted in altered cytokine release may also have been involved. In support of the significance of our findings, a recent investigation discovered CD73-deficient people with severe artery calcifications. It is still unknown whether these people have altered arteriogenesis [Schrader J. et al, 2013].

Nowadays, it is more widely acknowledged that strategies for a biology-based optimization and individualization of radiotherapy should take into account knowledge about the immune system's ability to modulate the radiation response, in addition to knowledge about tumor-promoting mutations, tumor heterogeneity, tumor cell plasticity, and unfavorable gene expression profiles indicative of the individual radiosensitivity of tumor and normal tissues. Such a broad perspective will enable the future development of biologically optimized therapeutic strategies with acceptable safety profiles and long-lasting responses by combining the potential of high precision local radiotherapy, cytotoxic chemotherapy, molecularly targeted small molecule signal transduction inhibitors, and immunotherapy approaches. [Jendrossek V., 2019] The use of radiotherapy in combination with various immunotherapies, particularly immune checkpoint blockade immunostimulatory antibodies, and cancer vaccines, has drawn significant attention due to the observation that radiotherapy can help to reactivate anti-tumor immunity in immunogenic tumors or increase the potential of immunotherapy. Tumors, however, have developed reliable ways to evade immune surveillance, and therapy-induced enhancement of tumor immunity is countered by feed-back inhibition of immune activation in residual tumors, the mobilization of tissue regeneration mechanisms with tumor-promoting effects, or both [Jendrossek V., 2019]. By I slowing the growth and metastasis of lung tumors, (ii) boosting the radiation-induced activation of the antitumor immune response, (iii) limiting the immunosuppressive action of CD39/CD73 on circulating immune cells, and (iv) attenuating the immunosuppressive effect of CD39/CD73 on circulating immune cells, pharmacologic inhibition of CD73/adenosine signaling is an appealing approach to increase In addition, pharmacological manipulation of CD73, adenosine, or the four adenosine receptors may present chances to increase the capacity of combined radioimmunotherapy to develop effective and sustained responses with a

favorable safety profile. 2019 [Jendrossek V.] Cancer cells and host cells, including but not limited to a variety of immune cell types, that express CD73 produce an adenosine-rich TME that suppresses the immune system. The tumor-promoting effect of cancer cell-intrinsic CD73 is supported by emerging findings. CD73 principally acts through its enzymatic activity to encourage tumor growth and metastasis. In-depth research is needed to better understand CD73's role in carcinogenesis irrespective of its enzymatic activity. This will reveal new information on CD73's regulatory role in cancer. Co-targeting CD73 with different therapeutic drugs is a logical move because CD73 expression and activity seem to be affected by several therapies. Inhibition of CD73 is generally.

Based on this data, MPs may be used therapeutically to modify the function of cells that take up these exosomes and MPs as well as biomarkers of inflammatory pathways [Longhi M.S. et al., 2019]. Adenosine levels in the irradiated tumor are significantly increased after local RT, which may lessen the anti-tumor effects of RT that are mediated by the induction of immunosuppression. Patients with locally advanced rectal cancer may have better results when receiving RT in combination with CD73/adenosine axis blocking [Tsukui H. et al, 2020]. Malignant salivary gland tumors were found to overexpress CD73. Therefore, CD73 may function as a possible biological marker to distinguish between malignant and benign salivary gland cancers in addition to the existing prognostic factors. Additionally, according to the results of this study, lymph node metastases and CD73 expression were positively correlated. In order to anticipate the biological behavior of salivary gland malignancies, doctors may be able to use immunohistochemistry examination of CD73 [Ranjbar M-A. et al., 2019]. The course of inflammatory diseases in the GI tract and liver is determined by the balance of ATP and adenosine. However, there are still many unanswered concerns regarding how this equilibrium is controlled and might be addressed in various illness contexts [Longhi M.S. et al, 2019]. It may be possible to deploy purinergic-based therapeutics to modulate these reactions in the gut and liver, either alone or in combination with already available therapies. In order to promote healing in these significant acute and chronic inflammatory processes, such therapies will aim to restore and maintain immunologic tolerance [Longhi M.S. et al, 2019].

This study indicates that CD73 on T cells is essential for the repair of heart wounds following myocardial infarction. The underlying mechanism entails a significant rise in ATP/NAD and AMP hydrolysis, which is predominantly brought on by the overexpression of pyrophosphatases and CD73. It has also been established that T cell-derived CD73 inhibits proinflammatory/profibrotic cytokines via A2bR/A2aR-mediated autacoid feedback [Schrader J. et al., 2017]. Members of three families with symptomatic arterial and joint calcifications have NT5E mutations. This gene produces CD73, which changes AMP into adenosine and supports the idea that this metabolic pathway prevents the calcification of ectopic tissue. (National Institutes of Health funding provided by the National Human Genome Research Institute and the National Heart, Lung, and Blood Institute.) [St. Hilaire C. and others, 2011] The recently discovered ERCs are crucial for immunoregulation. For ERCs to effectively prevent transplant rejection and increase cardiac allograft survival, CD73 expression is essential. The findings of this study serve as the foundation for the exact practical application of ERCs in transplantation [Wang H. et al, 2020]. According to the specific inflammatory situation and the cell types involved, CD73 can have pro- or anti-inflammatory effects in the brain, which is a crucial factor for possible therapeutic applications of CD73 modulators in CNS inflammation. In 2019, Minor M. et al.

The findings of this research thesis give up new perspectives for understanding CD73/unique ecto-5'-nucleotidase's function in the brain relative to other 5'-nucleotidases. They demonstrate that CD73 activity properly regulates behaviors such as locomotion and social interactions. It is important to note that alterations in these behavioral patterns are endophenotypes of prevalent behavioral disorders including schizophrenia and autism. Additionally, alterations in adenosinergic signaling have previously been connected to psychiatric diseases including schizophrenia. The discovery that CD73 is a key modulator of adenosinergic signaling in the brain may open up new opportunities for drug treatment of behavioral disorders. In 2013, Kulesskaya N. et al.

The new results that microglial mobility and neuroinflammation are modulated by CD73-produced adenosine in Parkinson's disease models highlight the significance of nucleotide metabolism in the control of immune

responses under various physiological and pathological circumstances. Targeting the upstream nucleotide metabolic pathway that controls adenosine production to modulate neural-immune interactions and neuroinflammation-related machinery instead of just the downstream adenosine receptor-mediated signaling represents a promising therapeutic approach for delaying the progression of Parkinson's disease [Meng F. et al, 2019]. One of the most significant findings is that CD73 can be utilized to identify and transplant cell populations at specific stages of the photoreceptor lineage [Koso H. et al., 2009].

Inflammation is one of the many processes that adenosine, a purine nucleotide, controls in living things. The adenosinergic system, like it does in other tissues, controls the inflammatory environment in the retina. Regarding the development of therapeutic approaches to treat retinal illnesses with an inflammatory component, adenosine receptors are one essential target, but so are enzymes and transporters involved in the metabolism and transport of adenosine. The function of each adenosinergic system component, the stage of the disease, and the start and length of the treatment will all affect how well these medicines work. Promising results from a number of preclinical investigations provide insight into the part played by adenosine in inflammation in retinal and other illnesses. To fully understand the function of adenosine in inflammatory processes and apply this knowledge in clinical practice, however, a great deal more research is required. In 2020, Santiago A. R. et al.

To assess the targeted blockage of adenosine signaling as an alternative and successful future approach, clinical trials seeking to translate anti-CD73 techniques in melanoma treatment are needed [Passarelli A. et al, 2019]. Using soluble CD73 as a potential prognostic factor that might be used with other established indicators for clinical outcomes in patients with unresectable melanoma [Turiello R, et al, 2020] is a possibility. An appealing therapeutic target for reprogramming cancer cells and the tumor microenvironment to reduce antitumor immune cell evasion is CD73, a cell surface 5'nucleotidase that produces adenosine. [J.B. Harvey, 2020]. Our understanding of the function of ectoenzyme CD73 will be expanded by future research, which calls for a larger sample size, additional stratification by stage of the disease (early stage, remitting/relapsing form, and advanced stage), and functional assays of the capacity of these enzymes. There is accumulating clinical evidence that the CD73-adenosine pathway has a role in immunosuppression and angiogenesis, making it an attractive therapeutic target in RCC. Both clear cell and non-ccRCC malignancies express CD73 in large amounts, with de novo mRCC and sarcomatoid tumors having a tendency to express it more. Independent of stage and grade, higher expression was associated with worse DFS and OS in individuals with localized illness. Additionally, higher levels of immunosuppressive cell markers were linked to the expression of the CD73, CD39, and A2AR genes, and in the TCGA cohort, A2AR expression was tied with the angiogenesis signature. Our results are consistent with the expanding research into this mechanism in advanced RCC [Harshman C. et al., 2020]. In processes connected to cancer, CD73 plays a variety of roles. In the upcoming era of individualized cancer therapy, the relationship between CD73 overexpression and cancer subtype, prognosis, and patient therapeutic response has demonstrated the potential relevance of CD73 as a detectable biomarker. Additionally, CD73 may be a therapeutic target for the treatment of cancer due to its stimulatory influence on tumor growth and metastasis. In mouse tumor models, targeting CD73 treatment with an inhibitor or mAb has shown promising anticancer benefits. Combining CD73 inhibition with other immune-therapeutic medications (anti-CTLA-4 mAb, anti-PD1 mAb) appears particularly appealing. These findings offer a great chance to create anti-CD73 therapy for the treatment of some cancer patients. Future research that try to apply anti-CD73 therapy to clinical cancer patients can be anticipated, even though there is still much to learn in this area. Notably, despite the lack of adverse effects in anti-CD73 treated mouse models, various functions of CD73 in vivo necessitate careful consideration of the anti-CD73 therapy's potential hazardous risks before applying this therapeutic strategy to cancer patients [Zhang H-Z. et al., 2014]. In order to fully realize the potential of targeting these escape mechanisms, research should be focused on a number of areas in the future, such as the evaluation of CD73 expression levels in various cancer stages, the safety, efficacy, and mechanism of action of anti-CD73 monoclonal antibodies, the clarification of negative effects of CD73 blockade, the impact of CD73 inhibition in both CD73 positive and CD73 negative tumors, and targeted blockade of specific escape mechanisms. 2019 [Farhad Jadidi-Niaragh]. One study uses strong CD73 expression and bioactivity to convincingly highlight one of CAFs' previously unknown

immunosuppressive activities. Through the CAF/tissue-specific A2B pathway, this CAF-CD73hi state is dynamically controlled and enforced throughout tumor growth and therapy. In order to improve the currently proposed/ongoing tests of A2A and/or CD73-neutralization clinical trials, as well as other activate therapies that induce massive cell death1, it will be therapeutically necessary to simultaneously block the non-redundant CAF-A2B-CD73 circuit and ADO-A2A-dependent inhibition of immune activation [Yu M. et al., 2020]. This will alleviate the CAF-CD73-mediated immune checkpoint for ultimate and durable tumor control. Increased levels of the soluble version of CD73 (sCD73) in the blood may serve as a prognostic indicator for tumor hypoxia and tissue inflammation. 2018 [Chaloin L. et al]. Adenosine plays a key role in how the heart responds to transplantation, and raising peri-transplant adenosine levels lessens the negative effects of IRI and enhances graft outcomes. [Dwyer K.M. and others, 2014] Model showing the function of A2AR signaling and CD73-produced adenosine production in microglia-mediated neuroinflammation and neuronal degeneration. Meng F. et al. 2019,] The creation of powerful CD73 inhibitors and the combination of CD73 and A2AR inhibitors may have more noticeable impacts on the recovery of microglia function and the treatment of neurological disorders. In 2019, Meng F. et al.

Overall, the information acquired will help to direct the lead optimization phase, which could result in strong and specific CD73 inhibitors that can revive the anticancer immune response. There is an appealing therapeutic target for reprogramming cancer cells and the tumor microenvironment to reduce antitumor immune cell evasion is CD73, a cell surface 5'nucleotidase that produces adenosine. CD73 is an excellent therapeutic target for cancer therapy [Zhang B. et al, 2020] and further investigations should be carried out to expand the full potential of this crucial enzyme beyond solid tumours.

REFERENCING

List of all references available on a seprate documents and can be provided upon request.

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